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Mini-Review **Open Access**

A mini-review of Mpox management in low- and-middle-income countries: A systems-based approach integrating CDC emerging infectious disease modelling and genomic surveillance

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Abstract:

Mpox, previously known as monkeypox, is a viral illness caused by the monkeypox virus (MPXV), a species of the Poxviridae family, and genus Orthopoxvirus. The resurgence of Mpox, particularly in low- and middle-income countries (LMICs), underscores the need for a comprehensive systems-level analysis. The Centre for Disease Control and Prevention (CDC)'s Emerging Infectious Diseases (EID) modelling framework, combined with genomic surveillance, offers a robust approach to understanding and mitigating such outbreaks. Integrating these methods can enhance outbreak prediction, surveillance, and response strategies, particularly in resource-constrained settings. This review aims to identify systemic vulnerabilities and health systems gaps in control of Mpox in LMICs, opportunities for modelling Mpox within the CDC EID framework, and the role of genomic epidemiology in Mpox surveillance, especially in combination with the EID framework. We used a narrative review and followed a structured method designed to capture the range of interventions on EID modelling and genomic surveillance related to Mpox. Previous modelling results indicated that close-contact transmission within and between households were unlikely to result in a large number of Mpox clade I cases. Studies that modelled the impact of vaccination and sexual behaviour adaptations on Mpox cases found that initial decline in cases were likely caused by behavioural adaptation, but vaccination averted more cases overall and was key to hastening outbreak control. Studies revealed that the geographical pattern of Mpox reported in the Nigeria outbreak suggested a possible new and widespread zoonotic reservoir, but the animal reservoir remains unknown. Integrating genomic data into Mpox surveillance can facilitate early detection, identification of animal reservoir and inform targeted interventions. This review found that CDC successfully leveraged a SARS-CoV-2 wastewater surveillance system for Mpox and concluded that integrating the CDC's EID modelling and genomic surveillance can significantly enhance Mpox management in LMICs.

Keywords: Mpox, Emerging Infection, Modelling, Genomic Surveillance, Zoonotic outbreaks.

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Synthèse des mesures de prise en charge de la variole du singe dans les pays à revenu faible et intermédiaire: Une approche systémique intégrant la modélisation des maladies infectieuses émergentes et la surveillance génomique du CDC

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Résumé:

La variole du singe, également appelée Mpox, est une maladie virale causée par le virus de la variole du singe (MPXV), une espèce de la famille des Poxviridae et du genre Orthopoxvirus. La résurgence de la variole, notamment dans les pays à revenu faible et intermédiaire (PRFI), souligne la nécessité d'une analyse systémique globale. Le cadre de modélisation des maladies infectieuses émergentes (MIE) des Centres pour le contrôle et la prévention des maladies (CDC), associé à la surveillance génomique, offre une approche robuste pour comprendre et atténuer ces épidémies. L'intégration de ces méthodes peut améliorer la prévision, la surveillance et les stratégies de riposte aux épidémies, en particulier dans les contextes aux ressources limitées. Cette revue vise à identifier les vulnérabilités systémiques et les lacunes des systèmes de santé dans la lutte contre la variole dans les PRFI, les possibilités de modélisation de la variole dans le cadre des MIE des CDC, et le rôle de l'épidémiologie génomique dans la surveillance de la variole, notamment en combinaison avec ce cadre. Nous avons utilisé une revue narrative et suivi une méthode structurée conçue pour recenser l'ensemble des interventions en matière de modélisation des MIE et de surveillance génomique liées à la variole. Les résultats de modélisations antérieures indiquaient que la transmission par contact étroit au sein des ménages et entre eux était peu susceptible d'entraîner un grand nombre de cas de variole de clade I. Des études modélisant l'impact de la vaccination et des adaptations des comportements sexuels sur les cas de variole ont montré que la baisse initiale du nombre de cas était probablement due à l'adaptation comportementale, mais que la vaccination a permis d'éviter un plus grand nombre de cas et a joué un rôle clé dans l'accélération du contrôle de l'épidémie. Des études ont révélé que la répartition géographique de la variole observée lors de l'épidémie au Nigéria suggérait l'existence possible d'un nouveau réservoir zoonotique étendu, mais l'animal réservoir demeure inconnu. L'intégration des données génomiques à la surveillance de la variole peut faciliter la détection précoce, l'identification du réservoir animal et orienter les interventions ciblées. Cette analyse a montré que les CDC ont exploité avec succès un système de surveillance des eaux usées pour le SARS-CoV-2 afin de détecter la variole et a conclu que l'intégration de la modélisation des maladies infectieuses émergentes (MIE) des CDC et de la surveillance génomique peut améliorer considérablement la gestion de la variole dans les pays à revenu faible et intermédiaire.

Mots-clés: Variole, infection émergente, modélisation, surveillance génomique, épidémies zoonotiques.

Introduction:

Mpox, previously known as monkeypox, is a viral illness caused by the monkeypox virus (MPXV), a species of the Poxviridae family, and genus Orthopoxvirus (same genus as the variola virus that causes smallpox and the vaccinia virus that causes cowpox) (1,2). While related to smallpox, Mpox presents with less severe symptoms and is not as easily transmissible. The virus was first identified in monkeys in 1958, leading to its original name. The first human case was recorded in 1970 in the Democratic Republic of the Congo (DRC). The virus has two main clades; Clade I and Clade II. Clade I is historically found in Central Africa and is associated with more severe disease and higher fatality rates than Clade II. Clade II, prevalent in West Africa, was responsible for the global outbreak that began in 2022. Since October, 2023, a large-scale Mpox outbreak has emerged in DRC, and in March, 2024, the first individuals with Mpox were reported outside the endemic areas of the country (3,4)

The recent global resurgence of Mpox underscores the need for robust public health responses and improved surveillance. Studies have shown that MPXV is mutating at an exceptional rate for a DNA virus and its global spread is concerning, making genomic surveillance a necessity (5). Genomic surveillance, which involves analyzing the virus's genetic material, is crucial for tracking its evolution and spread, enabling researchers to identify changes and assess potential public health implications.

The 2024 Mpox outbreak in Africa prompted swift action, with the Africa Centres for Disease Control and Prevention (Africa CDC) and the World Health Organization (WHO) declaring it a public health emergency of continental security and international concern on August 13 and 14, 2024, respectively. Weak health systems, limited surveillance and diagnostic capabilities, and lack of access to vaccines have hindered control efforts, allowing the outbreak to persist. The situation is further complicated by the emergence of new cases in non-endemic countries, including Kenya, Morocco, Rwanda, and Uganda, as well as shifting transmission patterns (6).

The resurgence of Mpox, particularly in low-and-middle-income countries (LMICs) underscores the need for a comprehensive systems-level analysis. The Centres for Disease Control and Prevention's (CDC) Emerging Infectious Diseases (EID) modelling framework, combined with genomic surveillance, offers a robust approach to understanding and mitigating such outbreaks. Integrating these methods can enhance outbreak prediction, surveillance, and response strategies, particularly in resource-constrained settings (5,7–10). Therefore, this review aims to identify systemic vulnerabilities and health systems gaps in control of Mpox in LMICs, opportunities for modelling Mpox within the CDC EID framework, and the role of genomic epidemiology in Mpox surveillance, especially in combination with the EID framework.

Methodology:

A narrative review approach was employed to synthesize evidence from diverse sources on EID modelling framework and genomic surveillance for Mpox. The review followed a structured method designed to capture the range of interventions on emerging infectious disease modelling and genomic surveillance related to Mpox. Databases searched included PubMed, Lancet, ScienceDirect, Web of Science, Google Scholar and Scopus.

Search terms were formulated using Boolean operators to combine keywords. Only English-language studies were included, and there were no restrictions on publication date. Over 20 studies were reviewed following abstract/title and full text review, with evidence drawn across countries such as DRC, Burundi, Rwanda, Uganda, Kenya, Nigeria, China and the United States.

Mpox in LMICs: Systemic vulnerabilities and health systems gaps:

The LMICs face multifaceted challenges in managing Mpox outbreaks, including socioeconomic disparities, limited healthcare infrastructure, and political instability. Humanitarian crises and conflict settings further exacerbate these vulnerabilities, hindering effective disease surveillance and response. Other public health challenges include vaccine inequity, surveillance limitations, and stigma-related barriers to disease reporting and control as highlighted in Table 1 (6,11–14). Tackling these complex challenges demands a unified global effort that combines fair vaccine distribution, enhanced surveillance, stigma-free health education, and coordinated outbreak response strategies (15,16).

Modelling Mpox within the CDC EID framework:

The CDC's EID modelling framework provides tools to simulate disease transmission dynamics and assess intervention strategies. Applying this to Mpox involves understanding outbreak patterns, identifying surveillance triggers, and incorporating a 'One Health' perspective that considers human-animal-environment interactions (Fig 1). The epidemiological model framework for mpox has been established over the past few decades, and many models that capture human–human, and animal–human interactions have been explored (17).

Previous modelling results indicated that close-contact transmission within and between households were unlikely to result in a large number of Mox clade I cases. Household transmission clusters would most likely involve 10 or fewer Mpox clade I cases, with minimal spread between households (18,19). This has an implication on the relative importance of human-human transmission vis-à-vis animal-human transmission in the outbreak of Mpox.

Table 1: Health systems and other challenges faced by low-and-middle-income countries in controlling Mpox

Category	Challenges
Health systems	Fatigue in healthcare systems Inadequate resources Differential availability of vaccines and drugs Lack of adequate laboratory infrastructure and healthcare workers Weak disease surveillance systems Delayed response mechanisms Inadequate knowledge about mpox among health care workers
Political	Political instability and crisis Funding for WHO & member states, particularly for extensive needs in Africa
Sociocultural	Ethnic considerations Stigmatization
Socioeconomic	Low educational attainment Low-income households and size of households
Humanitarian crisis related	Mobility corridors Sites for displaced people Other mobile populations Cross-border communities
Miscellaneous	Nonspecific clinical features Diagnostic challenges Lack of knowledge about mpox among community members

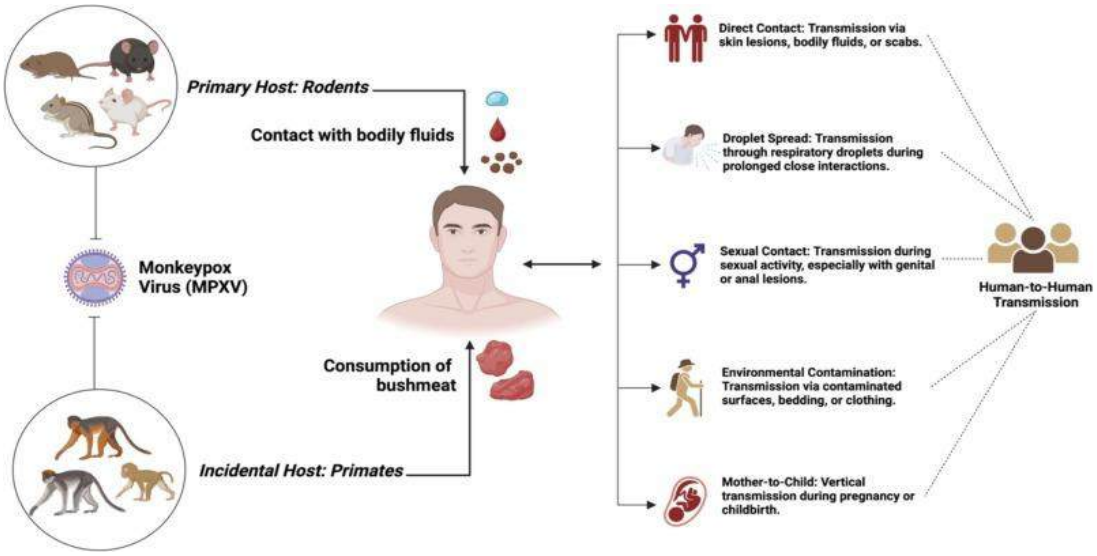

Fig 1: Interactions among human, animal reservoirs and environmental factors in Mpox transmission [Source: Verma et al.. (23)]

In addition, studies that modelled the impact of vaccination and sexual behaviour adaptations on Mpox cases in the USA during the 2022 outbreak found that initial declines in cases were likely caused by behavioural adaptation, but vaccination averted more cases overall and was key to hastening outbreak (20,21). A similar study in DRC informed the most efficient use of vaccines (22). These findings reinforce the role of modelling in identifying the most effective and also the most efficient interventions in the control of EIDs.

Role of genomic epidemiology in Mpox surveillance:

Genomic surveillance enables the identification of viral lineages, transmission chains, and mutation patterns. In LMICs, efforts like Nigeria's genomic sequencing initiatives have been pivotal in tracking diseases such as polio and Lassa fever. Studies revealed that the geographical pattern or Mpox reported in the Nigeria outbreak suggested a possible new and widespread zoonotic reservoir, but the animal reservoir remains unknown (24). Integrating genomic data into Mpox surveillance can facilitate early detection, identification of animal reservoir and inform targeted interventions. Furthermore, this is consistent with the 'One Health' approach which seeks to achieve a delicate balance between human, animal, and ecosystem health, acknowledging the intricate connections between the three domains.

Adopting a 'One Health' framework for disease surveillance, control, and prevention fosters a coordinated effort that brings together data from human, animal, and environmental health sectors, including pathogen genomics. This approach enables a comprehensive understanding of health outcomes and informs effective and efficient strategies for disease mitigation and prevention (25). This review found that CDC successfully leveraged a SARS-CoV-2 wastewater surveillance system for Mpox (26). Fig 2 is a flowchart depicting the integration of genomic data into surveillance systems.

Operationalizing a systems-based response:

Implementing a systems-based approach entails: (i) Community-based surveillance and mHealth tools: Leveraging mobile technology and community health workers to enhance real-time data collection and reporting(28); (ii) Workforce training and task-shifting: Empowering local healthcare providers through training and delegation of responsibilities to optimize response efforts (29); (iii) Data systems integration: Developing platforms that consolidate genomic and syndromic data for comprehensive analysis (25); and (iv) Cold chain and mobile labs: Innovations like mobile suitcase laboratories have been deployed in regions like the Democratic Republic of Congo (DRC) to facilitate rapid diagnostics (4).

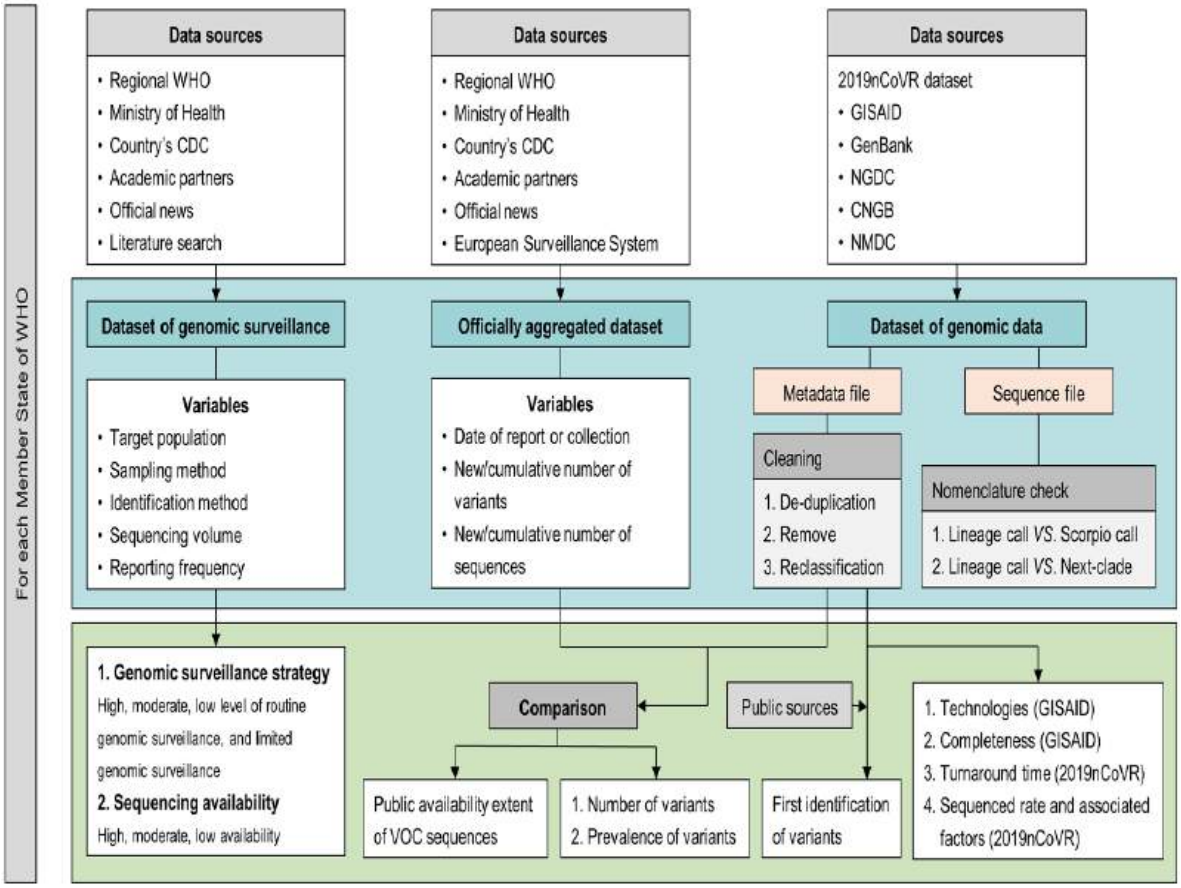

Fig 2: Flowchart depicting the integration of genomic data into surveillance systems [Source: Chen et al., (27)]

Recommendations for policy and practice:

These include investing in sequencing infrastructure and data governance to enhance local capacities for genomic analysis and establish protocols for data sharing and privacy; (ii) Embedding EID modelling into national preparedness plans with utilization of modelling tools to inform policy decisions and allocate resources effectively; (iii) Building regional data-sharing hubs and cross-border protocols to foster collaboration among neighboring countries to monitor and respond to outbreaks collectively; and (iv) Donor alignment and sustainability strategy with coordination of international funding to support long-term surveillance and response initiatives.

Conclusion:

Adopting a systems thinking approach that integrates CDC's EID modelling and genomic surveillance can significantly enhance Mpox management in LMICs. Such a multidisciplinary strategy addresses the complexities of zoonotic outbreaks and strengthens global health security.

Contributions of authors:

AB conceptualized the project, performed literature search and reviewed the manuscript; AEA wrote the first draft and did literature search; DD did literature search and combined the searches of all the authors.

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Mini-Review **Open Access**

The evidence gap: Vulvovaginal candidiasis research in African populations

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Abstract:

Vulvovaginal candidiasis (VVC) affects an estimated 70-75% of women at least once before menopause, and recurrent disease is estimated to affect approximately 138 million women annually worldwide. Within this global picture, sub-Saharan Africa carries a disproportionate share of non-*albicans Candida* infection and reported antifungal resistance, yet remains one of the least-studied regions for the mechanistic, immunological and therapeutic research now reshaping the field elsewhere. This mini-review summarizes the available African-specific evidence, identifies where that evidence stops short of matching the region's disease burden, and proposes concrete regional research priorities. The central challenge is not simply a shortage of prevalence surveys, of which a reasonable number now exist, but the near-total absence of African data from every mechanistic and translational thread currently advancing VVC science elsewhere: host immunogenetics, vaginal and gut microbiome characterization, novel antifungal evaluation, and vagina-specific diagnostic standards.

Keywords: Vulvovaginal candidiasis; Antifungal resistance; Non-*albicans Candida*; Research priorities; Host genetics; Africa

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Lacunes dans les données probantes: Recherche sur la candidose vulvo-vaginale dans les populations africaines

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Résumé:

La candidose vulvo-vaginale (CVV) touche environ 70 à 75 % des femmes au moins une fois avant la ménopause, et les récidives affecteraient près de 138 millions de femmes chaque année dans le monde. Dans ce contexte mondial, l'Afrique subsaharienne concentre une part disproportionnée des infections à *Candida* non-*albicans* et des résistances aux antifongiques, tout en demeurant l'une des régions les moins étudiées en matière de recherche mécanistique, immunologique et thérapeutique, une recherche qui redéfinit actuellement le domaine ailleurs. Cette mini-synthèse résume les données probantes disponibles spécifiques à l'Afrique, identifie les lacunes de ces données par rapport à la prévalence de la maladie dans la région et propose des priorités de recherche régionales concrètes. Le principal défi ne réside pas seulement dans la pénurie d'enquêtes de prévalence, bien qu'il en existe désormais un nombre raisonnable, mais dans l'absence quasi totale de données africaines concernant tous les axes de recherche mécanistiques et translationnels qui font progresser la science de la candidose vulvo-vaginale ailleurs dans le monde :

immunogénétique de l'hôte, caractérisation du microbiome vaginal et intestinal, évaluation de nouveaux antifongiques et normes diagnostiques spécifiques au vagin.

Mots-clés: Candidose vulvo-vaginale; Résistance aux antifongiques; *Candida* non-*albicans*; Priorités de recherche; Génétique de l'hôte; Afrique

Introduction:

Vulvovaginal candidiasis remains one of the most common mucosal infections affecting women of reproductive age worldwide (1). Global surveillance and meta-analytic work over the past several years have substantially refined understanding of its pathogen spectrum, host determinants and treatment landscape (2,3,4). A recurring finding in this literature, however, is that the populations carrying the heaviest burden of non-*albicans Candida* (NAC) infection and antifungal resistance are also the populations from which the least primary research originates (5).

This is not a minor gap. If host genetics, vaginal microbiome composition, and the distribution of infecting *Candida* species differ meaningfully between the settings where mechanistic research is generated and the settings that carry a disproportionate share of the global disease burden, therapeutic strategies developed elsewhere cannot be assumed to translate without direct testing.

What African data currently show:

Hospital-based studies from several African countries have documented both the scale of the problem and its species-level texture. A Nigerian tertiary-hospital cohort identified douching as a common modifiable risk factor alongside a high overall prevalence of symptomatic disease (6). An Ethiopian cohort found that non-*albicans* species accounted for more than 40% of culture-confirmed isolates, a substantially higher proportion than is typically reported in North American or European series (7).

A systematic review and meta-analysis of 41 studies from sub-Saharan Africa, comprising over 15,000 participants, estimated a pooled VVC prevalence approaching one-third of screened women and confirmed that susceptibility testing was available for only a minority of the underlying studies (5). Most recently, a Ghanaian tertiary-care case series documented antifungal-resistant *Candida albicans* directly implicated in recurrent disease, underscoring that resistance, not only species distribution, is now a live clinical concern in the region (8). These studies establish the scale of the challenge; they do not, on their own, explain it.

Where the evidence stops

Four gaps stand out as particularly consequential. First, host-genetic susceptibility research in VVC has been conducted almost entirely in populations of European ancestry. We are aware of a single genome-wide association study extending this work to an African population, conducted in Nairobi, Kenya, which identified candidate susceptibility loci for recurrent disease (9). This is a valuable precedent, but it stands alone, and its candidate loci have not yet been mechanistically connected to any of the immune pathways implicated in the broader pathogenesis literature.

Second, host-immune correlates of colonization versus symptomatic disease, an active area of investigation elsewhere, remain minimally characterized in African cohorts. One study of cervical immune activation in colonized South African adolescents found a distinct pattern of T-helper-17-like cell activation associated with asymptomatic carriage (10), and a 2025 scoping review synthesizing immunological data from Uganda, Zambia, South Africa and Kenya found a coordinated inflammatory cytokine signature but noted that the underlying primary literature remains sparse and heterogeneous in methodology (11). Whether the immune determinants of symptomatic disease identified in non-African cohorts operate similarly here is simply unknown.

Third, the microbiome data underpinning newer probiotic and microbiome-directed strategies derive almost exclusively from non-African human cohorts and animal models, and evidence for commercially available *Lactobacillus* preparations in preventing VVC remains inconsistent even in the populations where it has been tested (12). Traditional fermented foods common across West African diets, such as *'ogi'* and *'ugba'*, contain lactobacilli with plausible but uncharacterized anti-*Candida* potential (13). To our knowledge, none of these specific strains has been assayed for antifungal activity or tested within a microbiome-directed therapeutic framework, despite their obvious appeal as a low-cost, locally accessible intervention.

Fourth, the newer systemic antifungals now changing first-line practice elsewhere were evaluated primarily in North American and European trial populations, and their cost places

them outside routine reach in most African health systems (14). Meanwhile, standardized susceptibility testing itself has a validity problem specific to VVC: reference broth microdilution protocols are performed at neutral pH, while the vaginal environment is acidic, and susceptibility results can differ substantially under vaginally-relevant pH conditions (15,16). No antifungal agent used in VVC has a validated, vagina-specific susceptibility breakpoint anywhere, African data included.

Proposed priorities:

We propose four concrete, achievable priorities for African clinical microbiology research in this area. First, multicentre surveillance that pairs species identification with antifungal susceptibility testing as standard practice, rather than as an occasional add-on, building on the Ghanaian and Ethiopian precedents already established (7,8).

Second, extension of host-genetic and immune-correlate research beyond the single existing Kenyan cohort, ideally through multi-site African consortia that can achieve adequate sample sizes for genome-wide approaches (9).

Third, laboratory characterization of traditional fermented-food-derived lactobacilli for anti-*Candida* activity, as a direct, low-cost translational step building on existing food-microbiology literature (13).

Fourth, inclusion of African clinical sites in future trials of susceptibility breakpoint validation and novel antifungal evaluation, rather than post-hoc extrapolation from data generated elsewhere, alongside expanded molecular diagnostic capacity already emerging in some regional laboratories (17).

Conclusion:

The African evidence base for VVC is not empty, but it is structurally incomplete: strong on prevalence, thin on mechanism, and almost absent from the trials and cohort studies that will determine whether new diagnostic and therapeutic advances actually work in the populations that need them most. Closing this gap will require African-led, African-sited research at every level, from genomics to trial design, rather than continued reliance on evidence generated elsewhere and extrapolated after the fact.

Contributions of authors:

PCUO conceived the review concept and designed the study framework. SCE conducted the literature search and data extraction. PCUO synthesized the evidence and drafted the initial manuscript. INN critically revised the manuscript for intellectual content. All authors reviewed and approved the final version of the manuscript.

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Authors declare no conflicts of interest.

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Review Article **Open Access**

A review of vaccine adjuvants in modern immunology: Mechanisms, classes, and advances in next-generation vaccine design

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Abstract:

Vaccines are biological preparations that stimulate the immune system to prevent infectious disease without causing illness. By delivering weakened or inactivated pathogens, purified antigens, or genetic material encoding specific proteins, vaccines induce antibody production and generate memory B and T cells, enabling rapid, protective responses upon later exposure. Vaccination is among the most effective public health measures, leading to the eradication of smallpox in human and rinderpest in ruminants, and control of many infectious diseases. Advances such as recombinant and mRNA platforms have broadened applications to emerging infections and cancer immunotherapy. Adjuvants enhance vaccine immunogenicity, particularly in subunit and inactivated formulations. They activate innate immune pathways, including pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), triggering inflammatory signaling and cytokine release. This promotes recruitment and maturation of antigen-presenting cells, especially dendritic cells, improving antigen presentation and T-cell activation. Adjuvants also shape adaptive immunity by directing T-helper cell polarization and strengthening cytotoxic T-lymphocyte responses. Major adjuvant classes include aluminum salts (alum), which primarily elicit strong antibody (T_{h2}) responses; emulsion-based systems such as MF59 and AS03, which enhance antigen uptake and promote balanced humoral and cellular immunity; and TLR agonists such as monophosphoryl lipid A and CpG oligodeoxynucleotides, which drive T_{h1}-biased responses. Saponin-based and liposomal formulations further augment both antibody and cellular immunity, supporting vaccines against complex pathogens and cancer. Ongoing advances in molecular immunology and nanotechnology are enabling next-generation adjuvants designed to achieve precise, durable, and safe immune protection, underscoring their central role in future vaccine development and global disease prevention.

Keywords: Vaccines, adjuvants, immunological mechanisms, challenges and future

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Adjuvants vaccinaux en immunologie moderne: Mécanismes, classes et avancées dans la conception des vaccins de nouvelle generation

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Résumé:

Les vaccins sont des préparations biologiques qui stimulent le système immunitaire afin de prévenir les maladies infectieuses sans provoquer la maladie. En administrant des agents pathogènes atténués ou inactivés, des antigènes purifiés ou du matériel génétique codant pour des protéines spécifiques, les vaccins induisent la production d'anticorps et génèrent des lymphocytes B et T mémoire, permettant des réponses rapides et protectrices lors d'expositions ultérieures. La vaccination figure parmi les mesures de santé publique les plus efficaces, ayant conduit à l'éradication de la variole et au contrôle de nombreuses maladies infectieuses. Les avancées telles que les plateformes recombinantes et à ARNm ont élargi les applications aux infections émergentes et à l'immunothérapie du cancer. Les adjuvants renforcent l'immunogénicité des vaccins, en particulier dans les formulations sous-unitaires et inactivées. Ils activent les voies de l'immunité innée, notamment les récepteurs de reconnaissance des motifs (PRR) comme les récepteurs Toll-like, déclenchant des signaux inflammatoires et la libération de cytokines. Cela favorise le recrutement et la maturation des cellules présentatrices d'antigènes, en particulier les cellules dendritiques, améliorant ainsi la présentation de l'antigène et l'activation des lymphocytes T. Les adjuvants modulent également l'immunité adaptative en orientant la polarisation des lymphocytes T auxiliaires et en renforçant les réponses des lymphocytes T cytotoxiques. Les principales classes d'adjuvants comprennent les sels d'aluminium (alun), qui induisent principalement de fortes réponses humorales (T_{h2}) ; les systèmes à base d'émulsions tels que MF59 et AS03, qui améliorent la capture de l'antigène et favorisent une immunité humorale et cellulaire équilibrée; ainsi que les agonistes des récepteurs Toll-like comme le monophosphoryl lipid A et les oligodésoxynucléotides CpG, qui stimulent des réponses de type T_{h1}. Les formulations à base de saponines et les systèmes liposomaux renforcent davantage l'immunité humorale et cellulaire, soutenant le développement de vaccins contre des agents pathogènes complexes et le cancer. Les progrès continus en immunologie moléculaire et en nanotechnologie permettent le développement d'adjuvants de nouvelle génération conçus pour assurer une protection immunitaire précise, durable et sûre, soulignant leur rôle central dans la conception future des vaccins et la prévention mondiale des maladies.

Mots-clés: Vaccins, adjuvants, mécanismes immunologiques, défis et perspectives d'avenir

Introduction:

Vaccines are biological preparations designed to stimulate the immune system to recognize and defend against specific infectious agents, such as bacteria, viruses, or toxins. They work by introducing an antigen either a weakened or inactivated form of a pathogen, a fragment of it (such as a protein or polysaccharide), or genetic material encoding it into the body without causing the disease itself (1). This controlled exposure triggers the immune system to produce antibodies and activate immune cells, including memory B and T lymphocytes. As a result, if the vaccinated individual later encounters the actual pathogen, the immune system can respond rapidly and effectively, preventing illness or significantly reducing its severity (2).

Vaccination is one of the most successful and cost-effective public health interventions in history. It has led to the eradication of smallpox, near-eradication of polio, and dramatic reductions in diseases such as measles, diphtheria, tetanus, and whooping cough. Beyond individual protection, vaccines contribute to herd immunity, whereby high levels of immunization in a population reduce the spread of infectious diseases, protecting vulnerable individuals who cannot be vaccinated (3). With advances in biotechnology, modern vaccines including recombinant, vector-based, and mRNA platforms continue to expand the scope of disease prevention, addressing emerging infections and even non-communicable diseases such as cancer (4). This review aims to provide a comprehensive overview of vaccines, examining their mechanisms of action, types, and public health impact, while highlighting recent advances and future directions in vaccine development.

Methodology:

This is a narrative review of the scientific literature designed to provide a comprehensive synthesis of existing knowledge on vaccine adjuvants, their mechanisms of action, types, and safety profiles. Relevant data were obtained from peer-reviewed scientific literature sourced from major electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. Additional information was gathered from authoritative reports by global

health organizations such as the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC).

The search strategy involved the use of key terms including "vaccine adjuvants," "immunological mechanisms," "aluminum salts," "emulsion adjuvants," "TLR agonists," and "vaccine safety," with the use of Boolean operators, "AND" and "OR", to refine the results. Only articles published in English were considered, with priority given to recent and high-impact studies. The review primarily covers literature published between 2000 and 2025, while also incorporating seminars of earlier studies where necessary to provide historical context. Articles were screened for relevance based on titles, abstracts, and full-text evaluation, and selected findings were synthesized qualitatively to present a coherent and up-to-date overview of the field.

Immunological mechanisms of adjuvant action:

The mechanisms of adjuvant action begin with the activation of the innate immune system, which serves as the foundation for effective adaptive immunity. Most adjuvants function by stimulating pattern recognition receptors (PRRs) expressed on innate immune cells such as dendritic cells, macrophages, and monocytes. These receptors including Toll-like receptors (TLRs), NOD-like receptors (NLRs), and others recognize molecular patterns associated with pathogens or cellular stress. Engagement of these receptors triggers intracellular signaling pathways such as NF-κB and interferon regulatory factors (IRFs), leading to the production of pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNF-α) and type I interferons. This localized inflammatory response creates an immunostimulatory environment at the injection site that is essential for initiating strong immune responses (5,6).

A critical consequence of innate immune activation is the recruitment and maturation of antigen-presenting cells (APCs), particularly dendritic cells. Adjuvants enhance the uptake and processing of vaccine antigens and promote dendritic cell maturation, characterized by increased expression of major histocompatibility complex (MHC) molecules and costimulatory molecules such as CD80 and CD86 (7). Mature dendritic cells migrate to draining lymph nodes, where they present antigen-derived peptides to naïve T cells.

Effective T-cell activation requires three signals; antigen presentation via MHC (signal 1), co-stimulation (signal 2), and cytokine-mediated differentiation cues (signal 3) (Fig 1). By strengthening these signals, adjuvants prevent immune tolerance and ensure robust priming of antigen-specific T lymphocytes (7,8).

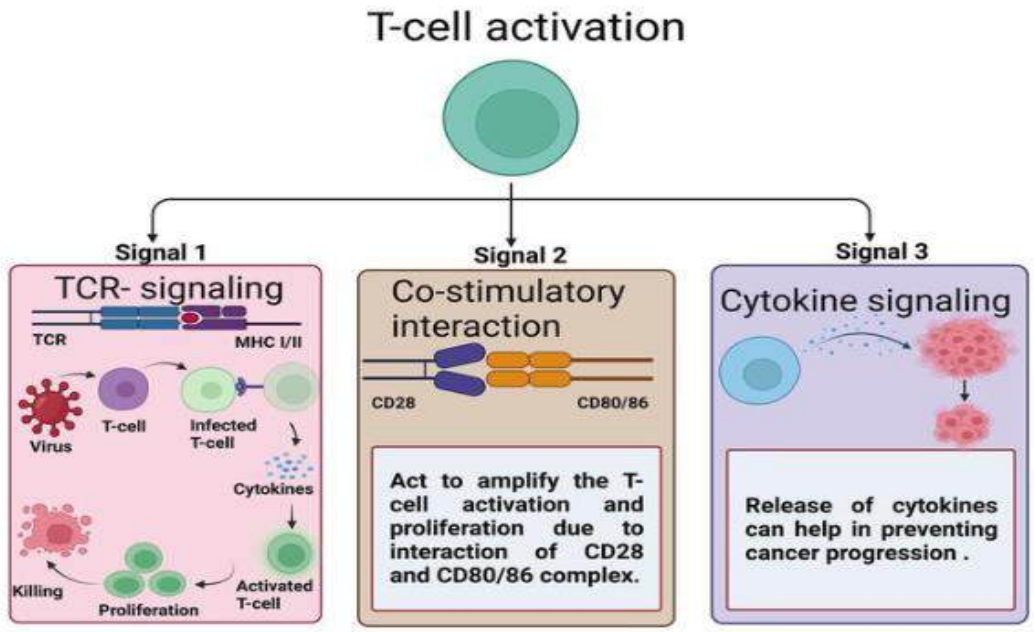

Fig. 1 Three signal hypotheses for T-cell activation: Signal 1-TCR-signaling; Signal 2-Co-stimulatory interaction between CD28 and CD80/86; Signal 3-Cytokine signaling (CC BY 4.0)

Adjuvants also shape the quality and polarization of adaptive immune responses. Depending on their composition and the innate pathways they activate, adjuvants can direct T-helper cell differentiation toward distinct functional subsets. For example, aluminum salts typically promote T_{h2}-biased responses that favor strong antibody production, whereas TLR agonists tend to induce T_{h1} responses associated with cellular immunity and cytotoxic T-cell activation. Some adjuvants facilitate cross-presentation, allowing exogenous antigens to enter the MHC class I pathway and stimulate CD8+ cytotoxic T lymphocytes, which are essential for protection against intracellular pathogens and cancer. Through these mechanisms, adjuvants tailor immune responses to match the nature of the targeted pathogen (9).

Finally, adjuvants enhance the magnitude and durability of humoral immunity by promoting germinal center formation and B-cell maturation. They support the development of T follicular helper (T_{fh}) cells, which assist B cells in undergoing affinity maturation, isotype switching, and differentiation into long-lived plasma cells and memory B cells. This result in higher antibody titers, improved antibody quality, and sustained immunological memory (10). By coordinating innate activation, antigen presentation, T-cell differentiation, and B-cell maturation, adjuvants act as essential immune modulators that transform weakly immunogenic antigens into highly effective and long-lasting vaccines (11).

Types of vaccine adjuvants:

Vaccine adjuvants can be broadly classified into several major categories based on their composition and mechanism of action. The oldest and most widely used adjuvants are mineral salts, particularly aluminum-based compounds such as aluminum hydroxide and aluminum phosphate, collectively referred to as alum (12). These adjuvants primarily enhance humoral immunity and are known to promote T_{h2}-type immune responses. Alum has been used for nearly a century in vaccines such as hepatitis B, diphtheria-tetanus-pertussis (DTaP), and human papillomavirus (HPV) vaccines. While highly effective at stimulating antibody production, aluminum salts are less efficient at inducing strong cellular (T_{h1} or cytotoxic T-cell) responses (13).

Another important category includes emulsion-based and combination adjuvants, such as MF59, AS03, and AS01. Emulsion adjuvants are typically oil-in-water formulations that enhance immune responses by recruiting antigen-presenting cells to the injection site and promoting antigen uptake. They are widely used in influenza vaccines, particularly for the elderly populations where stronger immune stimulation is required (6). Combination adjuvants, such as AS01 (which contains monophosphoryl lipid A and QS-21 in a liposomal formulation) are designed to activate multiple innate immune pathways simultaneously. These systems generate both strong antibody and cellular immune responses and are used in vaccines such as recombinant zoster vaccine (14).

A more targeted group of adjuvants includes pattern recognition receptor (PRR) agonists and saponin-based adjuvants. PRR agonists, such as monophosphoryl lipid A (a TLR4 agonist) and CpG oligodeoxynucleotides (TLR9 agonists), directly stimulate innate immune receptors, promoting T_{h1}-biased and cytotoxic T-cell responses. Saponin-based adjuvants, including QS-21 and Matrix-M, enhance both humoral and cellular immunity and are used in newer vaccines such as malaria and COVID-19 vaccines (15). In addition, emerging adjuvant technologies such as nanoparticle systems, liposomes, and STING agonists are being developed to provide more precise immune modulation. Together, these diverse types of adjuvants enable the design of vaccines tailored to specific pathogens and population needs (16).

Aluminum salts (Alum):

Aluminum salts are the most widely used adjuvants in human vaccines, commonly referred to collectively as "alum." The principal forms used in licensed vaccines are Aluminum hydroxide, Aluminum phosphate, and historically Potassium aluminum sulfate. Since their introduction in the 1920s, aluminum-based adjuvants have been incorporated into numerous vaccines to enhance immune responses to purified antigens that would otherwise be weakly immunogenic. Their long record of clinical use, established safety profile, and relatively low cost have made them foundational components of many routine immunization programs worldwide (17).

The mechanisms by which aluminum salts act as adjuvants are multifactorial. Traditionally, they were thought to function mainly through a "depot effect," in which the antigen adsorbed onto the aluminum particles is retained at the injection site and released slowly, prolonging immune exposure. More recent research shows that their activity also involves activation of innate immune pathways (18).

Aluminum salts promote local inflammation, recruit antigen-presenting cells such as dendritic cells, and enhance antigen uptake. They can stimulate the release of danger-associated molecular patterns (DAMPs) and activate inflammasome pathways, leading to cytokine production that supports adaptive immunity. Importantly, aluminum adjuvants tend to skew immune responses toward a T_{h2}-type profile, promoting strong antibody (humoral) responses rather than cytotoxic T-cell responses (19).

In terms of applications, aluminum salt adjuvants are included in many widely used vaccines, such as diphtheria vaccine, tetanus vaccine, hepatitis B vaccine, and certain formulations of the human papillomavirus vaccine. In these vaccines, aluminum salts adsorb protein antigens (such as toxoids or recombinant surface proteins), increasing their immunogenicity and enabling protective antibody titers with fewer doses or lower antigen quantities. They are especially effective for inactivated or subunit vaccines, where the pathogen is not live and requires immune enhancement to achieve adequate protection (12).

The characteristic features of aluminum salt adjuvants include their particulate nature, positive surface charge (depending on pH and formulation), and strong capacity for antigen adsorption through electrostatic interactions and ligand exchange. They are generally administered intramuscularly and are considered safe, with common side effects limited to mild local reactions such as pain, redness, or swelling at the injection site (20). Unlike newer adjuvants designed to elicit strong cellular immunity, aluminum salts are less effective at inducing robust T_{h1} or cytotoxic T lymphocyte responses, which limits their use in certain modern vaccines (e.g., some viral or cancer vaccines). Nevertheless, due to their stability, safety, affordability, and proven efficacy in stimulating durable antibody responses, aluminum salt adjuvants remain central to contemporary vaccinology (21).

Emulsion-based adjuvants:

Emulsion-based adjuvants are vaccine adjuvants formulated as mixtures of two immiscible liquids typically oil and water stabilized by surfactants to enhance immune responses to antigens. They are usually oil-in-water (O/W) emulsions, where small droplets of oil are dispersed within an aqueous phase. Prominent examples include MF59, AS03, and montanide ISA 51. MF59 and AS03 are squalene-based O/W emulsions widely used in human vaccines, whereas montanide formulations are often water-in-oil (W/O) emulsions used in veterinary and experimental vaccines. These systems are designed to improve immunogenicity, particularly for subunit or inactivated vaccines that may otherwise elicit weak immune responses (22).

The mechanisms of emulsion-based adjuvants involve both innate immune activation and enhancement of antigen delivery. Unlike traditional depot-forming adjuvants that simply retain antigen at the injection site, O/W emulsions such as MF59 actively recruit immune cells. They induce local production of chemokines and cytokines, which attract monocytes, macrophages, and dendritic cells to the injection site (23). These antigen-presenting cells (APCs) then uptake the antigen more efficiently and migrate to lymph nodes, where they prime T and B lymphocytes. Components such as squalene and α-tocopherol (in AS03) can further amplify immune signaling pathways, leading to stronger antibody titers and improved cellular immunity. The small droplet size and large surface area of the emulsion enhance antigen dispersion and interaction with immune cells (24).

Emulsion-based adjuvants are widely applied in human and veterinary vaccines. MF59 has been used in licensed influenza vaccines, particularly for older adults, to enhance immunogenicity against seasonal strains. AS03 gained prominence during influenza pandemics, including H1N1, due to its capacity to induce strong and cross-reactive immune responses with antigen-sparing effects (25). In veterinary medicine, Montanide emulsions are commonly used for vaccines against parasitic and viral infections in livestock. These adjuvants are especially valuable in pandemic preparedness, dose-sparing strategies, and in populations with weaker immune responses, such as the elderly. They are also under investigation for emerging infectious diseases and therapeutic cancer vaccines (26).

The characteristic features of emulsion-based adjuvants include their physical stability, biocompatibility (particularly when using metabolizable oils like squalene), and capacity to induce balanced humoral and cellular immunity (27). Oil-in-water emulsions are generally less reactogenic than older water-in-oil formulations, which were associated with stronger local inflammation and granuloma formation. They are typically manufactured with controlled droplet sizes (often in nanometer range) to ensure consistency, safety, and efficacy. While they may cause mild local reactions such as pain or swelling at the injection site, they have demonstrated strong safety profiles in licensed

vaccines (28). Overall, emulsion-based adjuvants represent a significant advancement in vaccine formulation by combining immunostimulatory capacity with acceptable tolerability and broad applicability (12).

Toll-like receptor (TLR) agonists:

Toll-like receptor (TLR) agonist adjuvants are immunostimulatory substances that enhance vaccine efficacy by specifically activating Toll-like receptors, a family of pattern recognition receptors expressed on innate immune cells such as dendritic cells, macrophages, and B cells. These receptors recognize conserved pathogen-associated molecular patterns (PAMPs), thereby initiating rapid immune activation. Notable examples of TLR agonists include monophosphoryl lipid A (a TLR4 agonist), CpG oligodeoxynucleotides, and imiquimod. Each of these molecule mimics microbial components to safely trigger innate immune signaling without causing full-blown infection, making them powerful tools in modern vaccine design (29,30).

The mechanism of TLR agonist adjuvants centers on receptor-specific activation of intracellular signaling cascades. Upon binding to their respective TLRs, these agonists stimulate adapter proteins such as MyD88 or Toll-interleukin receptor (TIR)-domain containing adapter-inducing interferon-β (TRIF), which then activate series of cytoplasmic kinases, leading to subsequent activation of transcription factors like nuclear factor kappa B (NF-κB) and interferon regulatory factors (IRFs). This results in the production of pro-inflammatory mediators (cytokines, chemokines), and type I interferons (Fig 2) (31).

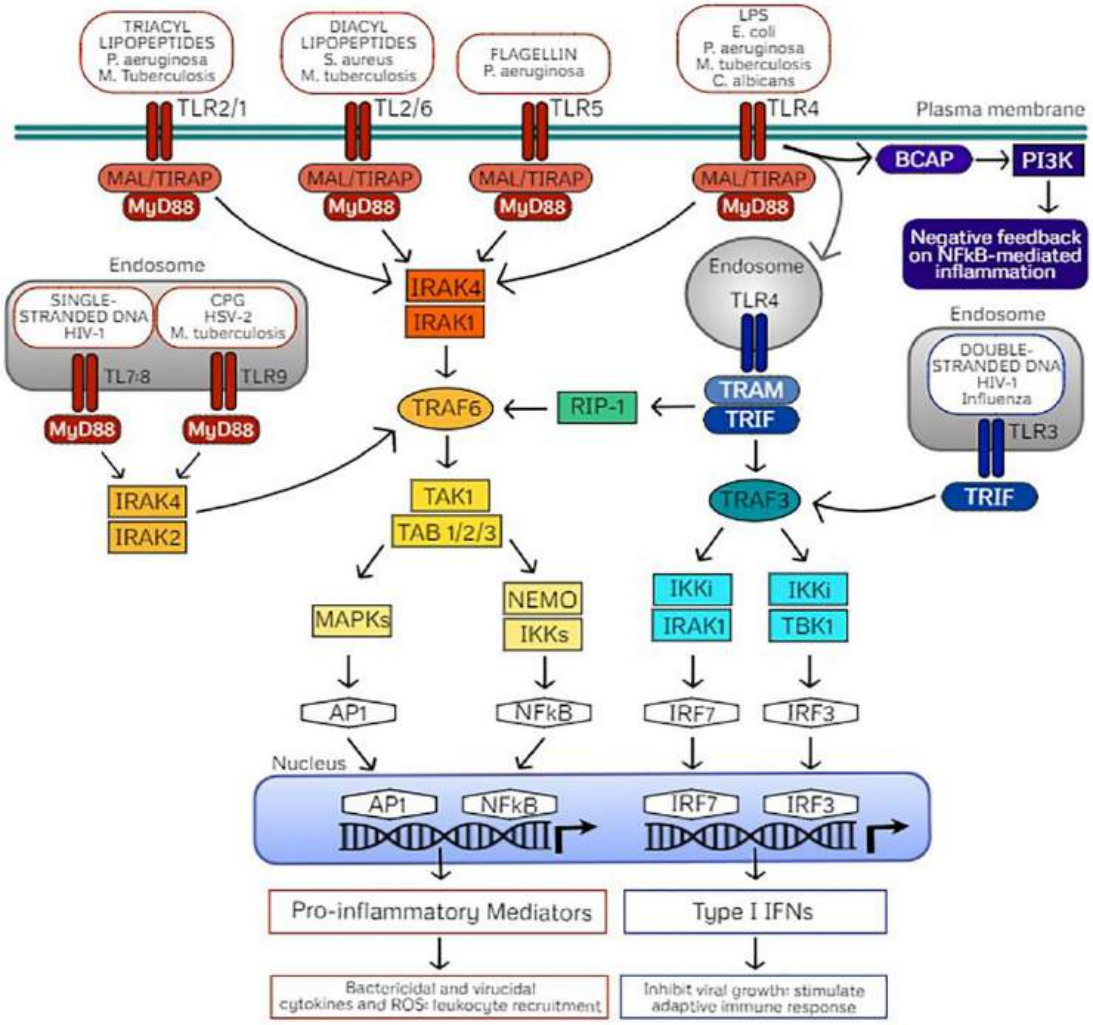

Fig 2: TLR signaling pathways mediated through MyD88- or TRIF-dependent cascades (31).

Consequently, antigen-presenting cells upregulate co-stimulatory molecules (e. g., CD80/CD86) and enhance antigen processing and presentation to T cells. For example, monophosphoryl lipid A promotes a balanced T_{h1}-biased immune response via TLR4 signaling, while CpG oligodeoxynucleotides stimulate strong T_{h1} and cytotoxic T-lymphocyte responses through TLR9 activation. This targeted engagement bridges innate and adaptive immunity efficiently (32).

In terms of application, TLR agonists are widely incorporated into licensed and experimental vaccines. Monophosphoryl lipid A is a component of the AS04 adjuvant system used in certain human vaccines, including those targeting viral infections. CpG oligodeoxynucleotides have been approved in specific hepatitis B vaccines to improve sero-protection rates, particularly in populations with weaker immune responses (33). Although primarily used topically for certain dermatological conditions, imiquimod has demonstrated adjuvant properties in therapeutic vaccine strategies and cancer immunotherapy. Beyond infectious diseases, TLR agonists are being explored in cancer vaccines and allergy immunotherapy due to their ability to skew immune responses toward desired T_{h1} or cytotoxic profiles (34).

The characteristic features of TLR agonist adjuvants include high specificity, potent immune activation, and the capacity to tailor immune polarization. They are often used in combination with other adjuvants (such as aluminum salts or emulsions) to create synergistic effects. Compared with traditional adjuvants, TLR agonists provide a more mechanistically defined mode of action, allowing rational vaccine design. However, because they strongly stimulate innate immunity, careful dose optimization is required to balance efficacy and reactogenicity (35). Overall, TLR agonist adjuvants represent a sophisticated generation of immune enhancers that leverage molecular insights into host–pathogen recognition to produce robust, durable, and targeted immune protection (29).

Saponin-based adjuvants:

Saponin-based adjuvants are immunostimulatory compounds derived primarily from plant glycosides known as saponins, which possess amphiphilic (both hydrophilic and hydrophobic) properties. These natural compounds are commonly extracted from the bark of the Chilean soapbark tree, *Quillaja saponaria* (36). One of the most studied purified saponins is QS-21, which has been incorporated into several advanced vaccine formulations. Saponins can also be formulated into more complex structures such as ISCOMs (immune-stimulating complexes), which combine saponins with cholesterol and phospholipids to create cage-like nanoparticles. Due to their potent immunostimulatory capacity, saponin-based adjuvants are widely investigated for both infectious disease and cancer vaccines (37).

The mechanisms of saponin-based adjuvants involve multiple immunological pathways. They enhance antigen uptake by antigen-presenting cells (APCs), promote dendritic cell maturation, and stimulate cytokine production. A key feature is their ability to induce strong cellular immunity, particularly cytotoxic CD8+ T-cell responses, which are essential for combating intracellular pathogens and tumors (38). QS-21 and related saponins are known to promote both T_{h1}-type immune responses, characterized by interferon-γ production and robust antibody production. Mechanistically, they may activate inflammasomes and facilitate antigen cross-presentation through membrane interaction and pore-forming activity. Their amphiphilic structure allows them to associate with cell membranes, enhancing antigen delivery and immune cell activation (37).

In terms of application, saponin-based adjuvants have been incorporated into licensed and experimental vaccines. QS-21 is a key component of the AS01 adjuvant system used in vaccines such as the malaria vaccine and certain herpes zoster vaccines. These formulations have demonstrated high efficacy by generating strong humoral and cellular immune responses (39). Beyond infectious diseases, saponin adjuvants are widely explored in therapeutic cancer vaccines due to their capacity to stimulate cytotoxic T lymphocytes. They are also under development for veterinary vaccines and emerging infectious diseases, particularly when a balanced and durable immune response is required (36).

The characteristic features of saponin-based adjuvants include their potent immunogenicity, ability to stimulate both arms of adaptive immunity (humoral and cellular), and compatibility with nanoparticle delivery systems. However, crude saponin extracts can be reactogenic or hemolytic, which is why purified fractions like QS-21 and formulated systems such as ISCOMs are preferred for human use. They are typically used at carefully optimized doses to minimize local and systemic side effects. Overall, saponin-based adjuvants represent a highly effective class of modern vaccine enhancers, valued for their strength, vers-

atility, and suitability for next-generation vaccine platforms (39).

Liposomal and combination adjuvants:

Liposomal adjuvants are vaccine delivery systems composed of phospholipid bilayer vesicles (liposomes) that encapsulate or adsorb antigens and immunostimulatory molecules. Their structure mimics biological membranes, allowing efficient fusion with or uptake by antigen-presenting cells (APCs). A prominent example is AS01, which contains monophosphoryl lipid A and QS-21 incorporated into liposomes. Another example is CAF01, a cationic liposomal formulation designed to induce strong cellular immunity (40). Liposomes can vary in size, charge (neutral, cationic, or anionic), and composition, allowing customization of immune responses. Their versatility makes them suitable for delivering protein subunits, peptides, nucleic acids, and even mRNA-based antigens (41).

The mechanisms of liposomal adjuvants involve enhanced antigen delivery, protection from degradation, and immune activation through co-delivered immunostimulants. Liposomes facilitate efficient uptake by dendritic cells and macrophages, promoting antigen processing and presentation via both MHC class I and II pathways. Cationic liposomes, in particular, form electrostatic interactions with negatively charged cell membranes, improving cellular uptake and depot formation at the injection site. When combined with immunostimulatory molecules such as monophosphoryl lipid A (a TLR4 agonist) or QS-21 (a saponin), liposomal systems can synergistically enhance cytokine production, dendritic cell maturation, and T-cell activation. This dual function serving as both a delivery vehicle and an immune enhancer distinguishes liposomal adjuvants from simpler adjuvant systems (42).

Combination adjuvants integrate two or more immunostimulatory components to achieve broader and more potent immune responses than single agents alone. For example, AS04 combines aluminum salts with monophosphoryl lipid A to enhance both antigen presentation and innate immune activation. Such systems are used in licensed vaccines against infectious diseases and are being explored for cancer immunotherapy and emerging pathogens (43). Characteristically, liposomal and combination adjuvants provide balanced humoral and cellular immunity, dose-sparing effects, and adaptable immune polarization (e. g., T_{h1}-biased responses). While formulation complexity and stability considerations require careful manufacturing control, these advanced adjuvant platforms represent a significant evolution in rational vaccine design, offering enhanced efficacy with acceptable safety profiles (44).

Emerging and experimental adjuvants:

Emerging and experimental adjuvants represent the next generation of immune-enhancing systems designed to improve vaccine efficacy, precision, and durability. Unlike traditional adjuvants such as aluminum salts, these novel platforms often target specific innate immune pathways or use advanced delivery technologies. Examples include cyclic GMP-AMP (a STING agonist), poly I:C (a TLR3 agonist), and CpG 1018 (45). In addition, nanoparticle-based systems, virus-like particles (VLPs), and RNA-based platforms are being investigated as both delivery vehicles and intrinsic adjuvants. These approaches aim to generate stronger cellular immunity, cross-protection against variants, and long-lasting memory responses (46).

The mechanisms of emerging adjuvants often involve precise activation of innate immune sensors such as STING, RIG-I–like receptors, and various Toll-like receptors. For example, cyclic GMP-AMP activates the STING pathway, leading to robust type I interferon production and enhanced dendritic cell maturation. Poly I:C mimics viral double-stranded RNA and stimulates TLR3, inducing antiviral cytokines and promoting T_{h1}-skewed immune responses (47). Many nanoparticle-based adjuvants enhance lymphatic trafficking and improve antigen cross-presentation, facilitating cytotoxic CD8+ T-cell activation. Some experimental systems are designed to co-deliver antigen and immune stimulators within the same particle, ensuring synchronized uptake by antigen-presenting cells. This targeted immune modulation allows fine-tuning of immune polarization, whether toward antibody-dominant (T_{h2}), cell-mediated (T_{h1}), or balanced responses (48).

Applications of emerging and experimental adjuvants span infectious diseases, cancer immunotherapy, allergy desensitization, and personalized vaccines. They are particularly valuable for subunit, peptide, and nucleic acid vaccines that lack intrinsic immunogenicity. In oncology, STING agonists and nanoparticle-based systems are being evaluated to stimulate tumor-specific cytotoxic T lymphocytes (49). In pandemic preparedness, RNA vaccines inherently possess self-adjuvanting properties through innate immune recognition

pathways, offering rapid scalability and adaptability. Characteristically, these novel adjuvants emphasize specificity, modularity, and synergy with advanced vaccine technologies. Although many remain under clinical evaluation due to concerns about reactogenicity and manufacturing complexity, they represent a transformative frontier in vaccinology, aiming for safer, more potent, and highly targeted immune protection (50).

Safety profiles and side effects of vaccine adjuvants:

The safety profiles of vaccine adjuvants are evaluated through extensive preclinical testing, phased clinical trials, and continuous post-licensure surveillance to ensure that their benefits outweigh potential risks. Historically used adjuvants such as aluminum salts (alum) have demonstrated decades of safe use in millions of doses worldwide, primarily causing mild and transient local reactions such as pain, redness, or swelling at the injection site (51). Modern adjuvants including emulsions, liposomal systems, saponin-based formulations, and Toll-like receptor (TLR) agonists undergo rigorous toxicological assessment to evaluate local reactogenicity, systemic effects, biodistribution and long-term safety. Regulatory agencies require detailed evaluation of dose, formulation stability, and immunostimulatory intensity to minimize adverse effects while preserving efficacy (52).

Most adjuvant-related adverse events are mild to moderate and reflect the intended activation of the innate immune system. Common effects include localized inflammation, low-grade fever, fatigue, or muscle aches, which are typically short-lived and resolve without intervention. Rare but more significant reactions such as severe allergic responses are carefully monitored during clinical trials and pharmacovigilance programs (53). Importantly, modern adjuvants are often designed to reduce systemic exposure and limit excessive inflammatory signaling. For example, detoxified derivatives of microbial components are used instead of native toxins to maintain immunostimulatory capacity while minimizing toxicity. Dose optimization and formulation strategies, such as nanoparticle encapsulation or liposomal delivery, further improve tolerability by controlling how and where immune activation occurs (54).

Long-term safety monitoring is a critical component of adjuvant evaluation, involving global surveillance systems and large-scale epidemiological studies. Extensive data from licensed vaccines show no consistent evidence linking approved adjuvants to chronic autoimmune diseases or long-term systemic harm (51,55). The biological mechanisms of adjuvants primarily transient activation of innate immunity support their short-lived action rather than persistent immune dysregulation. Nonetheless, continuous research aims to refine adjuvant design to balance potency with safety, especially for vulnerable populations such as infants, pregnant individuals, the elderly, and immunocompromised patients. Overall, the safety profiles of vaccine adjuvants reflect careful scientific design, stringent regulatory oversight, and decades of real-world evidence demonstrating that their benefits in preventing infectious diseases far exceed their risks (56).

Challenges in adjuvant development:

The development of vaccine adjuvants faces multiple scientific and regulatory challenges that make the process both complex and high-stakes. One of the primary scientific hurdles is achieving the optimal balance between immunogenicity and safety. Adjuvants must be potent enough to enhance immune responses to weak or subunit antigens but must not induce excessive inflammation or systemic toxicity. Overstimulation of the immune system can lead to adverse events, including local tissue damage, fever, or, in rare cases, autoimmune reactions. Designing adjuvants that consistently elicit the desired type of immune response whether humoral, cellular, or balanced is particularly challenging for diseases requiring specific immune polarization, such as intracellular pathogens or cancer (12,17).

Another major challenge in adjuvant development is variability in immune responses across populations. Factors such as age, sex, genetics, nutrition, microbiome composition, and prior exposure to pathogens can significantly influence how an individual responds to a given adjuvant. For example, older adults may have immunosenescence that reduces vaccine responsiveness, while infants have developing immune systems that are more sensitive to inflammatory stimuli. This variability complicates dose selection, formulation optimization, and safety assessment during clinical trials. It also necessitates large, diverse study populations and carefully tailored clinical trial designs to ensure that the adjuvant performs safely and effectively across demographics (57,58).

Regulatory and manufacturing hurdles

add additional layers of complexity. New adjuvants must undergo rigorous preclinical toxicology studies, phased human clinical trials, and post-licensure surveillance to demonstrate safety, efficacy, and batch-to-batch consistency. Manufacturing adjuvants with stable physical and chemical properties such as emulsions, liposomes, or nanoparticle formulations requires specialized facilities, precise quality control, and scalable production methods (59). Furthermore, combination or experimental adjuvants must meet even stricter standards due to their novel mechanisms of action. Regulatory agencies are cautious with new adjuvants because even minor formulation changes can impact immunogenicity or reactogenicity, making approval processes lengthy and resource-intensive (60).

A final challenge is the lack of universally predictive preclinical models. Animal models often fail to fully replicate human immune responses, leading to discrepancies between preclinical and clinical results. This is particularly true for adjuvants that act on specific innate immune pathways, such as Toll-like receptors or STING, where receptor expression and signaling differ between species. As a result, developers must rely on a combination of in vitro human immune cell assays, computational modeling, and carefully designed clinical trials to bridge this gap (61). Overall, these scientific, regulatory, and logistical challenges mean that adjuvant development is a slow, expensive, and highly iterative process, but overcoming them is essential for advancing vaccines against challenging or emerging infectious diseases (62).

Future perspectives of adjuvants:

The future of vaccine adjuvants is poised for transformative advances, driven by a deeper understanding of immunology, molecular biology, and materials science. Next-generation adjuvants aim to provide highly tailored immune responses, enhancing both efficacy and safety. One major direction is the rational design of adjuvants that selectively target specific innate immune receptors, such as TLRs, STING, or RIG-I–like receptors, to elicit precisely calibrated T_{h1}, T_{h2}, or cytotoxic responses. For example, synthetic TLR agonists and cyclic dinucleotide STING activators are being developed to boost cellular immunity for vaccines against intracellular pathogens or cancer, offering unprecedented specificity compared to traditional adjuvants (57).

Another emerging trend is the integration of advanced delivery platforms, including nanoparticles, liposomes, virus-like particles, and microneedle patches, which allow controlled release of antigens and adjuvants directly to immune cells. These technologies not only improve the magnitude and duration of immune responses but also reduce systemic reactogenicity (63). Combination adjuvants are likely to become more sophisticated, where multiple immunostimulatory signals are delivered simultaneously within the same formulation to achieve synergistic effects. Such systems can potentially overcome challenges posed by immunosenescence in elderly populations or hypo-responsive immune systems in certain patients (64).

Personalized and precision vaccinology is also expected to influence adjuvant development. Advances in genomics, transcriptomics, and immune profiling may allow vaccines to be tailored to individual immune profiles, optimizing both the type and strength of the response while minimizing adverse effects (65). Additionally, with the rapid expansion of mRNA and other nucleic acid vaccines, adjuvants that safely enhance these platforms by stabilizing RNA, improving delivery, or stimulating innate immunity will be crucial (66). Overall, the future perspectives of adjuvants revolve around precision, modularity, and synergistic design, combining immunological insights with innovative formulation technologies to create safer, more effective, and adaptable vaccines for a wide range of infectious diseases, cancers, and emerging global health threats (21).

Conclusion:

In summary, vaccine adjuvants play a critical role in enhancing immune responses, bridging the gap between antigen presentation and effective immunological memory. By modulating innate immunity and influencing the adaptive response, adjuvants improve vaccine efficacy and durability, which is especially important for subunit, recombinant, or inactivated vaccines that may be poorly immunogenic on their own. Understanding the mechanisms of action has led to the development of diverse adjuvant types, ranging from traditional aluminium salts to novel lipid-based and immunostimulatory compounds, each with distinct advantages and safety considerations. The careful evaluation of these adjuvants' immunological profiles ensures a balance between potency and tolerability, ultimately guiding safe and effective vaccine formulation.

Looking forward, the future of adjuvant research lies in designing highly targeted, pathogen-specific, and personalized adjuvants

capable of eliciting robust and durable protection with minimal side effects. Addressing challenges such as rare adverse events, regulatory hurdles, and variability in individual immune responses will be crucial to expanding their application. Advances in immunology, molecular biology, and nanotechnology hold promise for next-generation adjuvants that not only improve vaccine efficacy but also enable rapid responses to emerging infectious diseases. Ultimately, continued innovation in adjuvant science is pivotal to enhancing global vaccination strategies and strengthening public health outcomes worldwide.

Contributions of authors:

CPE conceptualized the study; MEN, CPE, CCA, and OVA conducted the literature search and data collection; CPE and MEN drafted the initial manuscript, while FE, OOO and JTA contributed to critical revision for intellectual content. All authors read and approved the final version of the manuscript.

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Authors declared that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Review Article **Open Access**

A scoping review of vulvovaginal candidiasis: Bridging the critical gaps toward precision diagnosis, treatment and recurrence prevention

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Abstract:

Vulvovaginal candidiasis (VVC) is among the most common mucosal fungal infections affecting women, with recurrent disease (RVVC) affecting an estimated 138 million women annually. Despite its frequency, major gaps remain in diagnosis, treatment and prevention. This scoping review identifies critical gaps across the VVC disease pathway and proposes a precision framework to transform management from episodic treatment toward prediction, targeted therapy and durable recurrence prevention. A comprehensive literature search was conducted in PubMed, Scopus, Web of Science, and African Journals Online using keywords related to VVC, antifungal resistance, microbiome, and treatment. Studies published between 1990 and 2026 addressing epidemiological, microbiological, immunological, diagnostic, therapeutic or translational aspects of VVC were included. Data were synthesized thematically. Major gaps identified include: the true burden of microbiologically confirmed symptomatic disease remains uncertain; symptoms are nonspecific while *Candida* colonization may occur without disease, creating a fundamental diagnostic problem; the biological mechanisms determining why some women remain asymptomatically colonized whereas others develop symptomatic or recurrent disease are incompletely understood; the vaginal microbiome and mycobiome are emerging as important determinants of disease phenotype, but most studies remain cross-sectional; *Candida* biofilms, strain-level variation and host inflammatory responses may contribute to persistence and recurrence, but their clinical significance requires better longitudinal investigation; and newer agents such as oteseconazole and ibrexafungerp have expanded therapeutic options, yet questions remain concerning comparative effectiveness, resistance, cost, access and long-term ecological effects. A precision framework integrating clinical phenotype, *Candida* biology, host susceptibility, microbial ecology and treatment history could transform VVC management. Priority areas include standardized disease definitions, longitudinal host–microbe studies, species- and strain-level characterization, antifungal susceptibility surveillance, microbiome and mycobiome research, patient-centred outcomes, and implementation studies in underrepresented populations.

Keywords: Recurrent vulvovaginal candidiasis; vaginal microbiome; mycobiome; antifungal resistance; precision medicine; antifungal stewardship.

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Une revue de portée de la candidose vulvovaginale: Combler les lacunes critiques vers un diagnostic, un traitement et une prévention des récidives de précision

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Résumé:

La candidose vulvovaginale (CVV) est l'une des infections fongiques muqueuses les plus courantes touchant les femmes, la forme récurrente (CVVR) touchant environ 138 millions de femmes chaque année dans le monde. Malgré sa fréquence, d'importantes lacunes subsistent dans le diagnostic, le traitement et la prévention. Cette revue de portée identifie les lacunes critiques tout au long du parcours de la maladie CVV et propose un cadre de médecine de précision pour faire évoluer la prise en charge d'un traitement épisodique vers la prédiction, un traitement ciblé et une prévention durable des récidives. Une recherche documentaire exhaustive a été menée dans PubMed, Scopus, Web of Science et African Journals Online à l'aide de mots-clés relatifs à la CVV, à la résistance antifongique, au microbiome et au traitement. Les études publiées entre 1990 et 2026 portant sur les aspects épidémiologiques, microbiologiques, immunologiques, diagnostiques, thérapeutiques ou translationnels de la CVV ont été incluses. Les données ont été synthétisées de manière thématique. Les principales lacunes identifiées comprennent : le fardeau réel de la maladie symptomatique confirmée sur le plan microbiologique demeure incertain ; les symptômes sont non spécifiques alors que la colonisation par *Candida* peut survenir sans maladie, ce qui crée un problème diagnostique fondamental ; les mécanismes biologiques déterminant pourquoi certaines femmes demeurent colonisées de manière asymptomatique alors que d'autres développent une maladie symptomatique ou récurrente sont mal compris ; le microbiome et le mycobiome vaginaux apparaissent comme des déterminants importants du phénotype de la maladie, mais la plupart des études demeurent transversales ; les biofilms de *Candida*, la variation au niveau des souches et les réponses inflammatoires de l'hôte pourraient contribuer à la persistance et à la récidive, mais leur signification clinique nécessite une meilleure investigation longitudinale ; et de nouveaux agents tels que l'otéséconazole et l'ibrexafungerp ont élargi les options thérapeutiques, mais des questions demeurent concernant leur efficacité comparative, la résistance, le coût, l'accès et les effets écologiques à long terme. Un cadre de précision intégrant le phénotype clinique, la biologie de *Candida*, la susceptibilité de l'hôte, l'écologie microbienne et les antécédents thérapeutiques pourrait transformer la prise en charge de la CVV. Les domaines prioritaires comprennent la normalisation des définitions de la maladie, les études longitudinales hôte-microbe, la caractérisation au niveau des espèces et des souches, la surveillance de la sensibilité antifongique, la recherche sur le microbiome et le mycobiome, les résultats centrés sur la patiente, et les études de mise en œuvre dans les populations sous-représentées.

Mots-clés: Candidose vulvovaginale récurrente; Microbiome vaginal; Mycobiome; Résistance antifongique; Médecine de précision; Gestion raisonnée des antifongiques

Introduction:

Vulvovaginal candidiasis (VVC) is a common infection of the female lower genital tract caused predominantly by *Candida* species. Approximately 75% of women are estimated to experience at least one episode during their lifetime, although precise estimates vary considerably according to population, case definition and diagnostic methodology (1-4). The global burden is substantial. A recent global systematic review and meta-analysis included 114 studies and more than 1.2 million women, demonstrating the widespread occurrence of VVC while also highlighting substantial heterogeneity between studies (5). In sub-Saharan Africa, a systematic review of 41 studies involving 15,723 participants estimated a pooled prevalence of 33%, with *Candida albicans* accounting for 71.7% of identified isolates and non-*albicans Candida* (NAC) species accounting for approximately 24% (6).

Recurrent VVC constitutes a particularly challenging phenotype. Denning et al., (7) estimated that approximately 138 million women experience RVVC annually, with the burden projected to increase as the global female population grows. Other epidemiological studies have demonstrated considerable variation in the estimated probability of recurrent disease following an initial episode (8,9). Yet the field faces an important paradox. VVC is common and familiar to clinicians, but some of its most fundamental questions remain unanswered. Why does *Candida* colonization produce symptoms in some women but not others? Why do some women develop repeated episodes despite apparently appropriate therapy? Why does suppressive treatment control recurrence while failing to provide durable cure in many women? Why do some women experience persistent symptoms despite negative or inconclusive microbiological findings? Why is antifungal resistance becoming an increasing concern in a disease that has historically been highly responsive to azole therapy?

These questions indicate that VVC should no longer be considered simply a localized fungal infection. Contemporary evidence supports a multifactorial model involving *Candida* biology, host response, vaginal microbial ecology, environmental exposures and treatment

history (10-15). This review therefore focuses not merely on what is known about VVC, but on unresolved gaps in the epidemiology, diagnosis, and treatment of VVC, and the research required to bridge these gaps (Table 1).

Methodology:

This scoping review was conducted following the methodological framework outlined by Arksey and O'Malley (16) and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines (17). The review aimed to map the current evidence on vulvovaginal candidiasis and identify critical gaps in epidemiology, diagnosis, treatment and recurrence prevention.

Research question:

What are the critical gaps in the understanding, diagnosis, treatment and prevention of vulvovaginal candidiasis, and what research is needed to bridge these gaps?

Search strategy:

A comprehensive literature search was conducted in PubMed, Scopus, Web of Science, and African Journals Online (AJOL) using combinations of the following keywords: "vulvovaginal candidiasis", "recurrent vulvovaginal candidiasis", "*Candida albicans*", "non-*albicans Candida*", "antifungal resistance", "vaginal microbiome", "mycobiome", "biofilm", "diagnosis", "treatment", "oteseconazole", "ibrexafungerp", "fluconazole", "probiotics", "Africa", and "sub-Saharan Africa". The search was limited to studies published in English language between 1990 and 2026.

Inclusion criteria:

Studies were included if they addressed epidemiological, microbiological, immunological, diagnostic, therapeutic or translational aspects of vulvovaginal candidiasis. Both original research and review articles were considered. Studies were excluded if they focused solely on systemic candidiasis, non-vaginal forms of candidiasis, or did not address VVC.

Table 1: Important research gaps on vulvovaginal candidiasis and future research needs

Critical gap	Current challenge	Research needed
Disease definition	Heterogeneous case definitions	Standardized clinical–microbiological criteria
Colonization vs disease	Candida detection may not indicate causation	Biomarkers of pathogenic infection
RVVC phenotype	Recurrence treated as one condition	Biological phenotyping
Host susceptibility	Predictors poorly understood	Prospective host-factor studies
Candida strain biology	Species-level data dominate	Longitudinal genomic studies
Microbiome	Mostly cross-sectional	Longitudinal bacteriome studies
Mycobiome	Understudied	Integrated fungal community profiling
Biofilms	Clinical relevance uncertain	In vivo/clinical correlation
Resistance	Surveillance incomplete	Standardized susceptibility surveillance
NAC VVC	Limited comparative evidence	Species-specific clinical trials
Treatment	Suppression ≠ cure	Durable recurrence-prevention strategies
New antifungals	Limited comparative data	Head-to-head effectiveness studies
Probiotics	Heterogeneous evidence	Strain-specific trials
Patient outcomes	Underrepresented	Quality-of-life/sexual-health endpoints
Africa/LMICs	Major evidence gaps	Multicentre longitudinal studies
Cost	Limited implementation evidence	Health-economic studies

NAC=Non-*albicans Candida*; VVC=Vulvovaginal candidiasis; RVVC= Recurrent vulvovaginal candidiasis; LMICs=Low-and-middle-income countries

Study selection:

All identified records were imported into EndNote and duplicates were removed. Two reviewers independently screened titles and abstracts against the inclusion criteria. Full texts of potentially relevant articles were then retrieved and assessed for eligibility by the same two reviewers. Disagreements were resolved through consensus or by consultation with a third reviewer. The study selection process is documented in a PRISMA-ScR flow diagram in Fig 1 (18).

Data extraction and synthesis:

A standardized data-charting form was developed to extract key information from included studies, including author, year, country, study design, population, VVC definition, species identified, antifungal susceptibility, treatment outcomes, microbiome findings, and identified gaps. Data were synthesized thematically, organized by key domains including epidemiology, pathogenesis, diagnosis, treatment, resistance, microbiome, host factors and research gaps.

Results

A total of 1,570 records were identified from four databases (PubMed, Scopus, Web of Science, and African Journals Online). An additional 35 records were identified through citation searching, bringing the total to 1,605 records. After removing 425 duplicates, 1,145 records were screened by title and abstract. Of these, 895 records were excluded as not relevant to VVC or outside the scope of the review. The remaining 250 full-text articles were assessed for eligibility, of which 128 were excluded for various reasons (not focused on VVC, no gap identification, duplicate/overlapping data, full text not available, or not in English). Ultimately, 122 studies were included in the final scoping review.

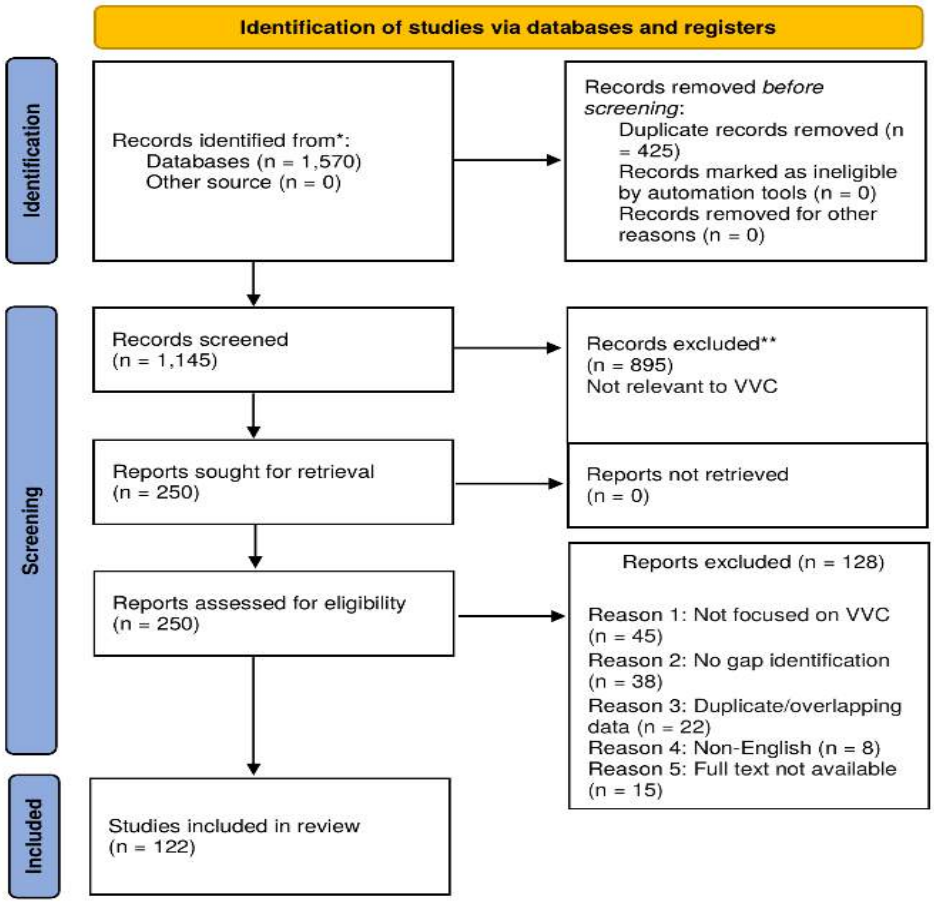

Fig 1: PRISMA-ScR flow diagram illustrating the study selection process for the scoping review

VVC evidence and critical gaps:

Epidemiology: A common disease with an uncertain denominator:

VVC is widely distributed across geographical and socioeconomic settings. *Candida albicans* remains the dominant species, but the relative contribution of NAC species varies considerably between populations (19,20). The apparent prevalence of VVC is strongly influenced by how the disease is defined. Studies may identify women with VVC according to self-reported symptoms, clinician diagnosis, microscopy, culture, molecular detection, positive *Candida* culture with symptoms, or positive *Candida* culture regardless of symptoms. These approaches do not identify the same population. The challenge is illustrated by the distinction between *Candida* colonization and symptomatic infection. *Candida* may be present in the vagina without causing disease, and therefore detection alone cannot establish causation (1,2). The 2026 global meta-analysis provides an important contemporary estimate of disease burden, but the substantial heterogeneity across studies demonstrates that the field still lacks a universally standardized epidemiological framework (5).

The critical gap here is the fact that the true burden of symptomatic, microbiologically confirmed VVC remains uncertain. Future epidemiological studies should clearly distinguish: (i) *Candida* colonization; (ii) symptomatic VVC; (iii) severe VVC; (iv) recurrent VVC; (v) persistent/refractory VVC; and (vi) non-infectious vulvovaginal conditions. Without this distinction, prevalence estimates remain difficult to compare and may substantially overestimate disease.

Recurrent VVC: The major unresolved clinical challenge:

RVVC is generally defined clinically as repeated symptomatic episodes over a defined period. The CDC currently uses three or more symptomatic episodes within one year, whereas older studies and some international literatures have used four or more episodes (1,8). This apparent simple difference illustrates a larger challenge, as definitions have not always been harmonized across studies. The global burden of RVVC is nevertheless substantial. Denning et al., (7) estimated approximately 138 million cases annually worldwide, with the highest prevalence in women aged 25--34 years.

RVVC is not merely repeated uncomplicated VVC (21,22), women with recurrent disease may experience: repeated clinical consultations, repeated laboratory testing, repeated antifungal exposure, treatment failure, anxiety concerning recurrence, sexual difficulties, disruption of work and daily activities, and substantial financial burden. Quality-of-life studies on RVVC demonstrate impairment across physical, psychological, social and sexual domains (23).

The critical gap here is that RVVC is insufficiently phenotyped. The label RVVC probably encompasses several biologically distinct conditions: persistent infection→relapse→reinfection→recurrent susceptibility→non-infectious mimics. Treating all of these as a single disorder limits therapeutic progress.

***Candida* colonization versus symptomatic disease:**

One of the most important diagnostic challenges in VVC is that *Candida* can be a commensal organism. The CDC notes that approximately 10-20% of women may harbour *Candida* or other yeasts vaginally without requiring treatment (1). Therefore, a positive *Candida* test does not automatically establish *Candida* as the cause of symptoms. This becomes particularly important with highly sensitive molecular tests.

A molecular assay may detect *Candida* DNA in a woman whose symptoms are caused by; bacterial vaginosis, trichomoniasis, vulvar dermatitis, lichen sclerosus, desquamative inflammatory vaginitis, genitourinary syndrome of menopause, irritant or allergic vulvitis or another condition (24). Consequently, increased analytical sensitivity is not synonymous with increased clinical specificity. The diagnosis of VVC should instead integrate symptoms + signs + laboratory evidence + species + clinical context.

The critical gap here is that there is no universally accepted biomarker that reliably distinguishes pathogenic *Candida* from asymptomatic colonization. This is one of the most important opportunities for future research. Potential biomarkers could include fungal burden, transcriptional activity, virulence-associated markers, host inflammatory signatures, metabolomic profiles, and microbial community signatures.

***Candida* species: Beyond *Candida albicans*:**

Candida albicans remains the dominant cause of VVC (19), However, NAC species are clinically important, particularly in recurrent, complicated and treatment-refractory disease (20,25). Common NAC species include *Candida glabrata (Nakaseomyces glabratus), Candida tropicalis, Candida krusei (Pichia kudriavzevii),* and *Candida parapsilosis*. A systematic review in sub-Saharan Africa found *C. glabrata (N. glabratus)*, *C. krusei* (*P. kudriavzevii*) and *C. tropi-*

calis among the leading NAC species (6). *Candida glabrata* (*N. glabratus*) is particularly challenging because it does not form the pseudohyphae or hyphae characteristic of *C. albicans* and may therefore be missed by conventional microscopy (1). NAC species may also have reduced susceptibility to commonly used azoles, complicating treatment (20,25). However, the presence of a NAC does not automatically establish causation. Some women with positive cultures are minimally symptomatic or asymptomatic (1,25).

The critical gap is that species identification is increasingly necessary, although species identification alone is insufficient. The future direction in the identification of agents of VVC should be species identification→susceptibility→ strain→clinical phenotype.

Risk factors and host susceptibility to VVC:

VVC has been associated with pregnancy, hormonal exposure, diabetes, antibiotic exposure, immunosuppression and other host and behavioural factors (19,26,27). However, these associations do not explain the majority of recurrent disease. The CDC notes that although RVVC can be secondary to frequent antibiotic use, diabetes or other host factors, most women with RVVC have no obvious predisposing condition (1). This is an important observation as it suggests that the conventional risk-factor model for VVC is incomplete. Host susceptibility may involve: epithelial barrier characteristics, innate immune responses, genetic susceptibility, inflammatory signaling, hormonal regulation, metabolic factors, and microbiome composition (10,28, 29,30,31).

The critical gap here is that we do not yet have a clinically useful model for predicting which women will progress from colonization or acute VVC to RVVC. Prospective cohorts could address this by identifying predictors before recurrence occurs.

Host immunity and inflammation:

VVC is unusual among *Candida* infections because it commonly affects immunocompetent women. The pathogenesis therefore cannot be explained by systemic immune deficiency (10,28,32). Studies of recurrent disease have demonstrated complex host immune responses involving local and systemic cellular immunity (29,30,31,33). Rare genetic human deficiencies in dectin-1 and caspase recruitment domain family member 9 (CARD9) signaling, which cause severe mucocutaneous candidiasis, illustrate how central innate pattern recognition is to antifungal host defense more broadly (34,35).

More recent work has emphasized the role of the vaginal epithelium as an active immunological interface. The epithelial response to *Candida* may involve pattern-recognition receptors binding, epithelial signaling pathways, neutrophil recruitment, inflammatory cytokines production, and tissue inflammation (10,28,29, 30,31). The toxin, candidalysin, secreted by hyphal forms of *Candida* is a well-characterized trigger of this epithelial danger response, activating NLRP3 inflammasome signaling and driving neutrophil recruitment at the vaginal mucosa (36,37). Importantly, inflammation may contribute substantially to symptoms. This creates a potential paradox, whereby the host response that attempts to control *Candida* may itself contribute to symptomatic disease.

The critical gap here is that the relationship between fungal burden and inflammatory severity remains poorly defined. Two women with similar *Candida* loads may experience very different clinical disease. Future studies should therefore measure; *Candida* quantity + *Candida* phenotype + host inflammatory response.

The vaginal microbiome:

The vaginal microbiome has become one of the most important areas of VVC research. *Lactobacillus*-dominated communities have traditionally been associated with vaginal health, but the relationship is more complex than a simple "*Lactobacillus* present versus absent" model (12,13,14,15,38,39,40). Studies of RVVC have identified differences in bacterial community composition, including alterations involving *Lactobacillus* species (13,14).

A study of women with RVVC identified reduced representation of some health-associated *Lactobacillus* species, including *Lactobacillus crispatus*, alongside increased *Lactobacillus iners*, and demonstrated interactions between *Lactobacillus* and *Candida* biofilms (14). Vaginal lactobacilli, notably *L. crispatus*, can directly inhibit *Candida* growth and hyphal transformation in vitro, providing a plausible mechanism for this association (41). Other studies have demonstrated considerable interindividual variability rather than a single characteristic "RVVC microbiome" (15).

The critical gap here is that there is no single microbiome signature that defines RVVC. This is important because it challenges the simplistic proposition that restoring *Lactobacillus* alone will necessarily cure recurrent disease.

Mycobiome: The missing fungal ecosystem:

Most microbiome research has focused on bacteria. However, the vaginal fungal community, the mycobiome, may also influence

disease. *Candida* exists within a broader microbial ecosystem and interacts with bacterial organisms, epithelial cells and host immune responses (12-15,42). One mycobiome study found that the identity of the infecting *Candida* species was itself associated with a characteristic bacterial community structure, suggesting the mycobiome and bacteriome shape one another rather than existing independently (43). This raises several questions; Does the composition of the vaginal mycobiome predict disease? Do non-*albicans Candida* species interact with *C. albicans*? Does repeated antifungal exposure alter fungal community structure? Does mycobiome disruption predispose to recurrence? These questions remain inadequately answered.

The critical gap here is the fact that VVC research has not yet fully integrated the bacterial microbiome and fungal mycobiome. Future studies should examine the bacteriome and mycobiome simultaneously, rather than treating *Candida* as an isolated pathogen.

Causality: Does dysbiosis cause VVC or result from it?

This is perhaps the most important conceptual problem in microbiome research. Most existing studies are cross-sectional. Therefore, when a woman with RVVC has an altered microbiome, we cannot necessarily determine whether dysbiosis → *Candida* overgrowth → VVC or *Candida* disease → inflammation → treatment → microbiome disruption. Repeated antifungal and antibiotic exposure may itself alter microbial communities.

The critical gap identified here is the absence of longitudinal microbiome studies, which are urgently required. The ideal study design should obtain samples before symptoms → during acute VVC → after treatment → during remission → at recurrence. This would allow researchers to identify microbial changes that precede rather than simply accompany disease.

Biofilms and persistence of *Candida*:

Candida biofilms are increasingly recognized as potential contributors to persistence and reduced antifungal susceptibility (44-47). Clinical isolates from women with recurrent disease show heterogeneous biofilm-forming capacity that correlates with reduced fluconazole sensitivity, and direct microscopic evidence has now demonstrated *Candida* biofilms on human vaginal epithelium itself, with tolerance to fluconazole at clinically relevant concentrations (48,49). A biofilm-competent mouse model further shows that biofilm formation generates antifungal-tolerant persister cells at higher frequency than planktonic growth (50).

Biofilms can alter fungal physiology, gene expression and antifungal response. A dynamic interkingdom model has also been proposed in which *Candida* and *Lactobacillus* interact within the vaginal environment (14). However, this evidence is not unanimous. One histologic study of vaginal biopsies found predominantly invasive, polymicrobial disease without clear biofilm structures, and cautioned against over-interpreting biofilm biology as the dominant mechanism of recurrence (51). This disagreement is itself informative, and much of the biofilm evidence overall remains derived from laboratory or limited clinical studies, a tension an earlier editorial on vaginal biofilms had already anticipated (52).

The critical gap here is the fact that the clinical importance of vaginal *Candida* biofilms remains uncertain. Future studies should determine whether biofilm-associated phenotypes predict treatment failure, recurrence, antifungal resistance, and persistence of particular *Candida* strains.

Persistence, relapse or reinfection?

When VVC recurs, clinicians often assume that *Candida* has returned. But returned *Candida* does not necessarily mean the same *Candida*. Recurrence could represent: (i) persistence of the original strain; (ii) evolution of the original strain; (iii) reinfection with a new strain; (iv) recolonization from another body site; or (v) recurrence of symptoms from a non-*Candida* condition. Traditional culture cannot adequately distinguish these possibilities. Early karyotyping work suggested that recurrence often reflects persistence and microevolution of a single strain rather than reinfection, but this was based on a small number of isolates and predates modern whole-genome approaches (53).

The critical gap is that strain-level longitudinal studies are lacking. Whole-genome sequencing of serial isolates could determine whether recurrent episodes arise from one persistent lineage or multiple independent acquisition events. This could fundamentally alter our approach to RVVC.

Diagnosis of VVC: Where the current model breaks down:

Clinical symptoms of VVC are common but non-specific. Pruritus, soreness, dyspareunia, external dysuria and abnormal discharge may occur with several vulvovaginal disorders (1). Microscopy remains useful, particularly because it can provide rapid evidence of yeast and inflammatory findings. Culture remains important in complicated disease because it permits

species identification and, where appropriate, susceptibility testing (1,54).

Molecular assays offer greater analytical sensitivity but create a new interpretive challenge: detection is not equivalent to causation. Head-to-head comparison of nucleic acid amplification testing against culture, Gram stain, and microscopy confirms substantially higher sensitivity for molecular detection, which sharpens rather than resolves this interpretive problem (55). For complicated VVC, current guidance recommends culture or PCR to confirm the diagnosis and identify NAC species (1).

The critical gap here is the lack of diagnostic algorithms that distinguish analytical detection from clinical disease, which are needed in this field. A future ideal diagnostic test should answer the following questions: Is *Candida* present in suspected VVC? Is the *Candida* active? Is the *Candida* pathogenic? Which species of *Candida* is involved? and is the *Candida* resistant to antifungal agents?

Antifungal resistance:

Azole resistance is becoming an increasingly important concern, particularly in recurrent or refractory disease (1,6,56,57). Resistance arises through convergent mechanisms including target-site mutation, efflux pump overexpression, and intrinsic reduced susceptibility in some non-*albicans* species, as documented for *C. glabrata* (*N. glabratus*) surveillance isolates (58,59). Standardized susceptibility testing itself has methodological gaps relevant to VVC. Specifically, reference broth microdilution protocols are performed at neutral pH, whereas the vaginal environment is acidic, and susceptibility results can differ substantially under vaginally-relevant pH conditions (60,61).

Literature from sub-Saharan African shows limited available susceptibility data on *Candida*. In the African meta-analysis, reported fluconazole resistance among *C. albicans* isolates varied substantially between countries, while susceptibility testing was available from only a subset of studies (6). The challenge is compounded by differences in susceptibility methods, breakpoints, species identification, previous antifungal exposure, and study populations.

The critical gap here is the fact that the global burden of clinically meaningful azole resistance in VVC is poorly quantified. Surveillance should therefore integrate antifungal exposure + species + susceptibility (minimum inhibitory concentration) + resistance mechanism + treatment outcome. Laboratory resistance without clinical correlation is insufficient. Emerging multi drug-resistant species outside the traditional NAC spectrum, such as *Candida auris*, underscore why resistance surveillance cannot remain static (62,63).

Antifungal stewardship:

Repeated empirical treatment can produce a cycle of symptoms → empirical antifungal → temporary improvement → recurrence → repeat empirical antifungal. The challenge is that persistent symptoms may not represent persistent *Candida*. The CDC recommends clinical reassessment for women whose symptoms persist after treatment or recur shortly after therapy (1). This is particularly important because unnecessary treatment may expose patients to adverse effects, increase cost, delay diagnosis of other vulvovaginal disorders; and contribute to antifungal selection pressure.

Antifungal stewardship should therefore become a component of VVC care, following the general antifungal stewardship principles already established for invasive candidiasis and other fungal disease priorities (64,65).

Treatment of uncomplicated and recurrent VVC:

Short-course topical or oral azole regimens remain highly effective for uncomplicated VVC (1). The major therapeutic challenge is therefore not that current drugs are ineffective against ordinary *C. albicans* VVC. The greater challenge is recurrence, complicated disease, NAC species and resistance. This distinction is important because it directs research toward the patients who remain underserved rather than simply developing new drugs for uncomplicated disease.

Suppressive fluconazole remains a major treatment strategy for RVVC. The landmark randomized trial by Sobel et al., (66) demonstrated that weekly fluconazole substantially reduced symptomatic recurrence during six months of maintenance therapy. At six months, 90.8% of women receiving fluconazole remained disease-free compared with 35.9% receiving placebo. However, after treatment was stopped, recurrence increased, demonstrating that suppression is not equivalent to durable cure (66). This finding remains central to the modern understanding of RVVC. The fundamental therapeutic challenge is therefore how to prevent recurrence after antifungal suppression ends.

Newer antifungal agents:

The therapeutic landscape of fungal infections has changed considerably, with the introduction of newer antifungal agents such as oteseconazole and ibrexafungerp.

Oteseconazole is a novel oral azole designed to selectively inhibit fungal CYP51. Phase 3 studies demonstrated substantial reduction in RVVC recurrence compared with placebo (67,

68). This built on earlier phase 2 dose-ranging and acute-VVC studies of the same compound (then designated VT-1161) that established proof of concept for once-weekly oral CYP51 inhibition (69,70). A subsequent randomized trial comparing oteseconazole directly with fluconazole in severe VVC, and a formal regulatory approval review, further contextualize its place in the therapeutic landscape (71,72).

In the VIOLET trials, oteseconazole demonstrated markedly lower recurrence rates through follow-up than placebo (68). This represents an important advance. However, several questions remain unanswered: Which patients should receive oteseconazole? Is it superior to prolonged fluconazole maintenance in real-world practice? What are the long-term resistance implications? How accessible is it in low-resource settings? What is its cost-effectiveness? and what is its impact on the vaginal mycobiome?

Ibrexafungerp is an oral triterpenoid glucan synthase inhibitor that provides a non-azole mechanism of action (73). Two pivotal phase 3 trials in acute VVC (VANISH 303 and VANISH 306) established its efficacy against placebo prior to the recurrence-prevention data from CANDLE (74,75). It demonstrated efficacy in VVC, and subsequent evidence has expanded interest in its role in recurrent disease (76). The CANDLE phase 3 study evaluated monthly oral ibrexafungerp for prevention of recurrent VVC, providing important evidence for a non-azole maintenance strategy (2). This is particularly relevant in an era of increasing concern about azole resistance. The critical gap here is the lack of comparative effectiveness research, which is needed. The key question is no longer simply "*Does the new drug work*?" It is "*Which drug works best for which phenotype, at what cost, with what long-term ecological and resistance consequences*?"

Non-*albicans Candida*: The persistent therapeutic challenge:

VVC caused by NAC is a particularly difficult therapeutic challenge because some species have reduced susceptibility to azoles and because the clinical significance of a positive culture may be uncertain (20,25). The CDC specifically emphasizes the need to exclude other causes of symptoms when NAC species are detected because some women may have minimal or no symptoms (1). This is a critical point. The treatment challenge is therefore not simply antifungal resistance. It is also diagnostic uncertainty + species biology + limited comparative clinical evidence.

The critical gap here is the fact that evidence-based species-specific treatment algorithms remain inadequate for NAC VVC. There is a need for randomized studies stratified by *Candida* species.

Probiotics and microbiome-directed therapy:

The idea of restoring a protective vaginal microbiome is attractive (77). However, clinical evidence has not consistently demonstrated that commercially available *Lactobacillus* preparations prevent VVC. A Cochrane systematic review of probiotics for VVC in non-pregnant women likewise found the evidence base too heterogeneous to support routine use (78). A randomized controlled trial by Pirotta et al., (79) found that oral or vaginal *Lactobacillus* preparations did not prevent post-antibiotic vulvovaginal candidiasis. This illustrates an important principle that biological plausibility does not equal clinical efficacy.

Different *Lactobacillus* strains have different properties, and probiotic preparations should not be considered interchangeable, a point developed at length in reviews of *Lactobacillus* derivatives for vaginal health (80). *In vitro* studies showed that specific strains such as *L. rhamnosus* GR-1 and *L. reuteri* RC-14 have measurable antifungal activity against *C. glabrata (N. glabratus)* isolates, while others show no such effect, underscoring that strain-level, not genus-level, evidence should guide selection (81,82).

The critical gap is the fact that microbiome therapy requires phenotype-specific clinical trials rather than generic probiotic studies. Future research should focus on investigating strain-specific interventions, live biotherapeutic products, vaginal rather than oral delivery, metabolite-based therapies, microbiome restoration, and bacteriophage or ecological approaches where appropriate.

Patient-centred outcomes:

VVC research has historically emphasized microbiological cure. Yet for women, the most important outcomes may be symptom resolution, absence of recurrence, sexual health, psychological wellbeing, ability to work, treatment convenience, and affordability. A study of women with RVVC demonstrated substantial impairment in physical, psychological, social and sexual domains (23).

The critical gap to fill here is the need for clinical trials to measure what outcome matters to patients. A woman who has a negative culture but persistent severe symptoms, is not necessarily experiencing therapeutic success. Likewise, a woman with microbiological clearance but major treatment-related toxicity may not

regard therapy as successful. Therefore, future trials should include validated patient-reported outcome measures.

Africa and other underrepresented populations:

The evidence base for VVC is geographically uneven. The systematic review of sub-Saharan African showed a substantial burden of VVC disease but also emphasized the limited availability of susceptibility data (6). Hospital-based studies from individual countries illustrate this unevenness directly: cohorts from Nigeria, Ethiopia, and Ghana each report differing species distributions and, in the Ghanaian case, direct evidence of antifungal-resistant *C. albicans* in recurrent disease, while a genome-wide association study from Kenya represents one of the only attempts to date to extend host-genetic susceptibility research beyond populations of European ancestry (83-86).

Immunological studies in African cohorts, including work characterizing cervical T_{h17}-like cell activation in asymptomatically colonized South African adolescents, further suggest that host-immune correlates of colonization versus disease may not be uniform across populations. An open question in a recent scoping review of immune responses in African women with vaginal candidiasis makes this explicit (87,88).

The critical gap here is the evidence-equity challenge in VVC research. The women most affected by diagnostic and therapeutic inequities may be the least represented in the evidence used to formulate guidelines. This creates an important research opportunity. African studies should move beyond repeated single-centre prevalence surveys toward multicentre surveillance, standardized diagnostics, species-level characterization, antifungal susceptibility testing, longitudinal RVVC cohort studies, microbiome/mycobiome studies, cost-effectiveness research, and implementation science. This is especially important because treatment strategies developed in high-income countries may not be directly transferable to settings where diagnostic facilities are limited, molecular assays are expensive, antifungal susceptibility testing is unavailable, newer antifungals may be inaccessible, and over-the-counter treatment is common.

Toward a precision model of VVC:

We propose that future VVC research should conceptualize disease as the interaction of five domains:

Domain 1: *Candida* species, strain, fungal burden, virulence, biofilm phenotype, and antifungal susceptibility.

Domain 2: Host genetics, immunity, epithelial biology, hormonal state, and diabetes/ metabolic status.

Domain 3: Microbial ecosystem: bacterial microbiome, mycobiome, microbial metabolites, and interkingdom interactions.

Domain 4: Environment and treatment: antibiotics, antifungal exposure, hormonal exposure, sexual and reproductive factors, and healthcare access.

Domain 5: Clinical phenotype: asymptomatic colonization, acute VVC, severe VVC, RVVC, and refractory disease.

These domains interact dynamically: Colonization → ecological/host perturbation→ symptomatic disease → treatment → ecological change → remission or persistence → recur rence. This model will provide a more useful framework for future research than treating *Candida* as an isolated pathogen.

What the ideal future study should look like:

A high-quality prospective RVVC cohort should recruit women before recurrence and follow them longitudinally. At baseline, assessment should include detailed clinical phenotype, metabolic assessment, medication history, antibiotic exposure, reproductive/hormonal history, and quality-of-life assessment. At each VVC episode, assessment should include microscopy, culture, species identification, susceptibility testing, *Candida* burden estimation, symptom severity, and inflammatory markers assessment. For selected cohorts, assessment should include bacterial metagenomics, mycobiome sequencing, metabolomics, host transcriptomics, and whole-genome sequencing of sequential *Candida* isolates.

Samples should be collected before disease → during disease → after treatment → during remission → at recurrence. Such studies could finally answer whether microbial or host changes precede recurrence or merely accompany it.

From association to prediction:

The ultimate goal of VVC research should be prediction. A clinically useful model might eventually estimate probability of symptomatic VVC, followed by probability of recurrence, followed by probability of treatment failure, and ultimately optimal treatment for the patient's biological phenotype. Such a model would represent a genuine transition toward precision medicine.

Antifungal stewardship in the precision era:

New antifungal agents should not simply replace older agents sequentially. The field should adopt an antifungal stewardship framework based on: (i) confirming the diagnosis; (ii)

identifying species when disease is complicated; (iii) assessing susceptibility when indicated; (iv) distinguishing recurrence from persistent non-infectious symptoms; (v) minimizing unnecessary exposure; and (vi) selecting newer agents for appropriate phenotypes. This is particularly important because novel agents may be more expensive and because their widespread use could eventually create new resistance pressures.

An eight-point priority research agenda:

We propose eight immediate priorities for VVC research:

Priority 1: Standardize definitions: Develop internationally accepted definitions for VVC, RVVC, refractory VVC, microbiological cure, clinical cure, and recurrence.

Priority 2: Develop better diagnostics: Move from *Candida* detection toward determination of *Candida*-associated disease.

Priority 3: Establish resistance surveillance: Create standardized international VVC antifungal susceptibility surveillance systems.

Priority 4: Build longitudinal cohorts: Follow women from colonization through disease and recurrence.

Priority 5: Integrate microbiome and mycobiome research: Study bacterial and fungal ecosystems together.

Priority 6: Investigate strain persistence: Use genomic methods to distinguish relapse from reinfection.

Priority 7: Compare therapeutic strategies: Conduct head-to-head trials of fluconazole, oteseconazole, ibrexafungerp and other emerging therapies in clearly defined phenotypes.

Priority 8: Address global inequity: Build research capacity and generate high-quality evidence from Africa, Asia, Latin America and other underrepresented populations.

Discussion

VVC has traditionally been approached as a straightforward infection: identify *Candida* and give an antifungal. This model works well for uncomplicated *C. albicans* VVC. However, it falls short for recurrent, complicated, NAC, or treatment-resistant disease. The real challenge is not just the organism, it is the relationship between the organism, the host, and the vaginal ecosystem. The colonization versus disease distinction is a key example. *Candida* can be present without symptoms, and women with symptoms may have conditions unrelated to *Candida* (1,25). Thus, a positive molecular or culture result should not automatically be taken as proof of causation.

Recurrence presents a similar problem. Weekly fluconazole can effectively suppress RVVC, but recurrence often returns after treatment stops (66). This shows that antifungal therapy can control symptoms without removing the underlying predisposition. The arrival of oteseconazole and ibrexafungerp is a meaningful advance (2,67,68,73,76) because it shows that new drugs can improve recurrence prevention. However, new drugs alone are not enough. If recurrence is driven by host factors, ecological changes, or persistent strains, then long-term control will need more than repeated antifungal courses.

The microbiome may hold part of the answer. However, current evidence does not support simple claims that "low *Lactobacillus* causes VVC" or that probiotics work for everyone (12-15,79). Instead, we need to learn which microbial communities are linked to which clinical patterns, and whether those communities are a cause, a consequence, or both. The African data highlight another issue: research inequality (6). A disease cannot be fully understood when large regions lack data on species distribution, resistance patterns, recurrence rates, or treatment outcomes. The future VVC research agenda must be both scientifically advanced and globally inclusive.

Conclusion:

Vulvovaginal candidiasis is common, clinically important and increasingly complex. The major challenge facing the management of VVC is no longer simply the availability of effective antifungal drugs. The deeper challenge is that we still have limited ability to determine who will develop symptomatic VVC disease, why disease recurs, why treatment fails, and how recurrence can be prevented sustainably. The critical gaps span the entire disease pathway, from epidemiological definition and diagnosis to *Candida* biology, host immunity, microbiome interactions, resistance, treatment and patient-centred outcomes.

Future VVC research should therefore move from *Candida* detection→antifungal treatment toward clinical phenotype + *Candida* species/strain + host susceptibility + microbial ecology + treatment history → precision diagnosis → individualized therapy → recurrence prevention. The field of VVC research should prioritize longitudinal, multicentre studies integrating clinical phenotyping, microbiology, antifungal susceptibility testing, genomics, microbiome/myco biome analysis and patient-reported outcomes.

The most important conceptual transition is from episodic treatment to precision pre-

vention. Ultimately, the goal should not simply be to develop more antifungal drugs. It should be to understand which woman is at risk, why she develops disease, which *Candida* phenotype is responsible, why treatment succeeds or fails, and which intervention can prevent recurrence without unnecessary antifungal exposure. That is the research agenda capable of moving VVC from a repeatedly treated common infection to a disease managed through precision diagnosis, targeted treatment and durable prevention.

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Contributions of authors:

PCUO conceived the review concept and designed the study framework. SCE conducted the literature search and data extraction. PCUO synthesized the evidence and drafted the initial manuscript. INN critically revised the manuscript for intellectual content. All authors reviewed and approved the final version of the manu script.

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Review Article **Open Access**

Evolving dynamics and antimicrobial resistance of *Vibrio cholerae* in Nigeria: An integrative epidemiological and microbiological review

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Abstract:

Background: Cholera remains recurrent in Nigeria, with outbreak location and magnitude varying over time in relation to population displacement, flooding, rapid urban growth, and constraints in water and sanitation services. Published microbiological and genomic studies describe circulation of seventh-pandemic *Vibrio cholerae* O1 El Tor lineages in Nigeria, with antimicrobial resistance (AMR) reported in multiple isolate series and occasional reports of non-O1/non-O139 strains.
Methodology: We conducted an integrative review combining Nigeria cholera surveillance reporting (January 1, 2017 to December 31, 2024) with microbiological and genomic literature relevant to Nigeria. State and year outbreak patterns were synthesized from national and partner reports. Microbiological characteristics, lineage assignments, and resistance mechanisms were summarized from peer reviewed literature and selected grey literature.
Results: Across 2017 to 2024, Nigeria experienced recurrent cholera surges with shifting geographic concentration. Northern states frequently contributed a large share of reported cases, particularly in conflict-affected and displacement settings, while recent reports also indicate risk in densely populated urban settlements. Microbiological evidence describes predominance of *V. cholerae* O1 El Tor, including atypical El Tor variants, with resistance patterns commonly involving trimethoprim-sulfamethoxazole and reduced fluoroquinolone susceptibility, linked to mobile genetic elements and target-site mutations in some isolate series. Genomic analyses that include Nigerian isolates place them within West African seventh-pandemic transmission clusters, including T12 and AFR12 lineage designations reported by study authors.
Conclusion: Available genomic literature supports sustained transmission of seventh-pandemic lineages and persistent AMR risks. Nigeria's cholera control planning for 2025 to 2029 provides an opportunity to link surveillance, diagnostics, WASH, and targeted vaccination, but impact will depend on implementation capacity and sustained financing.

Keywords: Evolving dynamics; Antimicrobial resistance; Integrative epidemiology; *Vibrio cholerae*

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Dynamique évolutive et résistance aux antimicrobiens de *Vibrio cholerae* au Nigéria : une revue épidémiologique et microbiologique intégrative

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Résumé:

Contexte: Le choléra demeure récurrent au Nigéria, et la localisation et l'ampleur des épidémies varient au fil du temps en fonction des déplacements de population. Inondations, croissance urbaine rapide et contraintes liées aux services d'eau et d'assainissement. Des études microbiologiques et génomiques publiées décrivent la circulation des lignées de *Vibrio cholerae* O1 El Tor, responsables de la septième pandémie, au Nigéria. Une résistance aux antimicrobiens (RAM) a été observée dans de nombreuses séries d'isolats, ainsi que des cas occasionnels de souches non-O1/non-O139.

Méthodologie: Nous avons réalisé une revue intégrative combinant les données de surveillance du choléra au Nigéria (du 1er janvier 2017 au 31 décembre 2024) avec la littérature microbiologique et génomique pertinente. Les schémas épidémiques par État et par année ont été synthétisés à partir des rapports nationaux et de ceux de nos partenaires. Les caractéristiques microbiologiques, l'identification des lignées et les mécanismes de résistance ont été résumés à partir de la littérature scientifique et de la littérature grise.

Résultats: De 2017 à 2024, le Nigéria a connu des flambées épidémiques de choléra récurrentes, avec une concentration géographique variable. Les États du nord ont souvent contribué de manière significative aux cas déclarés, en particulier dans les zones de conflit et de déplacement de population, tandis que des rapports récents indiquent également un risque dans les zones urbaines densément peuplées. Les données microbiologiques décrivent la prédominance de *V. cholerae* O1 El Tor, y compris des variants atypiques d'El Tor, avec des profils de résistance impliquant fréquemment le triméthoprime-sulfaméthoxazole et une sensibilité réduite aux fluoroquinolones, liés à des éléments génétiques mobiles et à des mutations du site cible dans certaines séries d'isolats. Les analyses génomiques incluant des isolats nigérians les situent au sein des clusters de transmission de la septième pandémie en Afrique de l'Ouest, notamment les lignées T12 et AFR12 rapportées par les auteurs de l'étude.

Conclusion: La littérature génomique disponible confirme la transmission soutenue des lignées de la septième pandémie et la persistance des risques de résistance aux antimicrobiens. Le plan de lutte contre le choléra au Nigéria pour la période 2025-2029 offre l'opportunité de coordonner la surveillance, le diagnostic, l'accès à l'eau, à l'assainissement et à l'hygiène (EAH) et la vaccination ciblée, mais son impact dépendra des capacités de mise en œuvre et d'un financement durable.

Mots-clés: Dynamique évolutive; Résistance aux antimicrobiens; Épidémiologie intégrative; *Vibrio cholerae*

Introduction:

Cholera is an acute diarrhoeal disease caused by toxigenic *Vibrio cholerae*, and it remains a major public health problem in many low- and middle-income settings. Global estimates suggest 1.3 to 4.0 million cases and 21,000 to 143,000 deaths annually, with a large proportion of reported burden occurring in sub-Saharan Africa (1). In 2024, a multi-country resurgence was documented, with substantial numbers of cases and deaths reported across multiple WHO regions (2). Nigeria has experienced recurrent outbreaks over the last decade, with substantial variation in timing, geography, and reported severity across years (3,4,5). Epidemiological risk is shaped by overlapping vulnerabilities, including population displacement and insecurity in parts of Northern Nigeria, flooding and disruption of water services, and rapid urban expansion with inadequate sanitation in informal settlements (6,7,8).

In parallel, microbiological and genomic studies show that outbreaks in Nigeria are largely associated with seventh-pandemic *V. cholerae* O1 El Tor lineages, including atypical El Tor variants described in Nigerian isolate collections, and antimicrobial resistance determinants linked to mobile genetic elements and quinolone resistance mechanisms in some datasets (9-13). Reports of non-O1/non-O139 *V. cholerae* associated with diarrhoeal illness also have implications for diagnostics, because O1-targeted rapid tests may not detect them (9,12).

Despite long-standing recognition of cholera risk in Nigeria, three gaps keep showing up across the literature and outbreak reporting. First, outbreak reports often provide large suspected case counts but limited microbiological confirmation and sparse reporting of isolate-level details. This makes it hard to distinguish true cholera from other causes of acute watery diarrhoea, and it weakens confidence in comparisons across states and years when case definitions, testing access, and reporting completeness vary (3,4,7).

Second, while several Nigerian studies describe serogroup distribution, virulence markers, and antimicrobial susceptibility patterns, the evidence is heterogeneous. Studies differ in sampling frames, culture methods, susceptibility methods, and interpretive standards, and they are often limited to single outbreaks or a small number of sites. This makes pooled estimates of resistance prevalence unreliable and creates uncertainty about how laboratory findings should inform empiric care and stewardship during outbreaks (9,10,13).

Third, genomic evidence relevant to Nigeria is growing but remains uneven over time. A substantial part of the genomic picture comes from regional or multi-country studies that include Nigerian isolates rather than continuous Nigeria-only sequencing. Even so, the available

phylogenomic work provides useful insights into seventh-pandemic lineage circulation, regional connectivity, and the likely movement of strains across West Africa, including lineage labels used by study authors such as T12 and AFR12 (11,12,14).

The practical challenge is converting these insights into operational tools that can be used during outbreaks, including specimen referral pathways, routine susceptibility testing linked to case management, and feasible sequencing or genotyping strategies integrated into surveillance (7,15). A synthesis that links the Nigeria Center for Disease Control and Prevention (NCDC) reporting with isolate and sequencing data is needed to interpret shifting hotspots and resistance risk. Nigeria's cholera control planning for 2025 to 2029 also makes the timing useful, because priorities such as targeted vaccination, water, sanitation and hygiene (WASH) investments, and improved surveillance can be better aligned when evidence is summarized in a way that is actionable for both public health and clinical decision-making (7,16).

This integrative review aims to synthesize epidemiological, microbiological, and genomic evidence on cholera in Nigeria, with a focus on the period January 1, 2017 to December 31, 2024 for outbreak epidemiology, and inclusion of earlier microbiological or genomic studies where needed to interpret circulating lineages and antimicrobial resistance patterns (17). Specifically, we address four questions; (i) What are the state-level and year-to-year patterns of reported cholera burden in Nigeria from 2017 to 2024 based on national surveillance reporting, and how do geographic concentrations shift across outbreak years (3,4,5)? (ii) What microbiological characteristics are reported from Nigeria outbreaks, including serogroups, biotype descriptors, and virulence markers, and what is the consistency of these findings across studies (10,14,18)? (iii) What antimicrobial susceptibility patterns and resistance mechanisms have been described in Nigerian *V. cholerae* isolate series, and what are the main limitations that affect interpretation for clinical guidance and stewardship (9,10,13)? (iv) What do genomic studies that include Nigerian isolates indicate about lineage assignments and regional clustering, and what are the implications for surveillance design and outbreak response in Nigeria (11,12,14)?

We summarize state-level outbreak patterns (2017 to 2024) and link them to what is known about circulating lineages and resistance in Nigeria, consolidates a state-by-year summary of surveillance-reported suspected cholera burden using a defined approach to selecting the last available sitrep for each year, summarizes microbiology and AMR evidence with attention to methods and comparability, situates Nigeria within the regional genomic context described by major Africa-wide and West Africa-focused sequencing work.

Materials and method:

We conducted an integrative review of cholera in Nigeria that combined surveillance-derived epidemiological reporting with microbiological and genomic evidence on *V. cholerae*. The epidemiological component covered January 1, 2017 to December 31, 2024. Microbiological and genomic studies published within the same period were included, and earlier studies were also included where needed to interpret circulating lineages and antimicrobial resistance patterns reported in Nigeria.

Evidence was compiled from three source streams; (i) Peer reviewed literature (PubMed, Web of Science, African Journals Online, and Google Scholar), (ii) National and global surveillance reports (NCDC weekly and annual cholera situation reports and outbreak updates, and World Health Organization cholera updates where applicable (2,4), and (iii) Grey literature and humanitarian or environmental reports (UNICEF and ReliefWeb reports and other humanitarian updates used to extract contextual information on flooding, displacement, WASH conditions, and response activities relevant to cholera transmission and control) (19,20,21).

Search strategy:

A structured search strategy was applied using combinations of organism, disease, geography, and thematic terms. Core terms included; "cholera", "*Vibrio cholerae*", "Nigeria", "outbreak", "epidemic", "antimicrobial resistance", "AMR", "genomics", "whole genome sequencing", "phylogeny", "lineage", "El Tor", "atypical El Tor", "non-O1", "non-O139", "integrative conjugative element", "ICE", "ctxB", "tcpA", "cholera vaccine", "oral cholera vaccine", and "WASH". Boolean operators (AND, OR) were used to restrict results to Nigeria and to prioritize microbiology, AMR, genomics, vaccine, and response literature. Reference lists of included articles and key Nigeria-focused papers were hand searched to identify additional eligible studies.

Database searches were last run on 20 February 2025. Google Scholar screening was restricted to the first 200 results sorted by relevance due to the high volume and variable quality of retrieval. For grey literature, targeted searches were performed within NCDC, WHO,

UNICEF, and ReliefWeb repositories using the same core keywords, and additional relevant documents were identified through citations and embedded links within retrieved reports. We included articles and reports available in English. Preprints were eligible if they provided sufficient methodological detail and Nigeria-specific extractable results. Where included, they were clearly identified as preprints and interpreted cautiously (14).

Eligibility criteria:

To map the breadth of evidence regarding *V. cholerae* in Nigeria, we included sources that met the following criteria: (i) Geographic relevance - studies reporting primary data from Nigeria at the national, state, local government area (LGA), or facility level; (ii) Content relevance - sources addressing one or more of the following key domains; (*a*) epidemiological indicators such as case counts (suspected/confirmed), mortality data (CFR), temporal trends, and geographic distribution; (*b*) microbiological and genomic characteristics such as serogrouping (O1, O139, non-O1/non-O139), biotyping, virulence determinants, antimicrobial susceptibility (AMR) phenotypes, and phylogenetic lineage assignments; (*c*) environmental and contextual drivers such as impact of flooding, population displacement, and WASH infrastructure on outbreak dynamics; (iii) Evidence type - adoption of inclusive approach including peer-reviewed primary research (observational, cross-sectional, and genomic studies), official surveillance reports (NCDC, WHO), and grey literature from reputable humanitarian agencies (UNICEF, ReliefWeb) to capture real-time outbreak data.

We excluded studies that did not provide extractable Nigeria-specific findings, duplicate reports of the same dataset, and opinion pieces or editorials that lacked primary data or systematic synthesis.

Case definitions and outcomes:

We extracted suspected and confirmed cholera counts as reported by each source, recognizing that definitions vary by reporting system and time period. Where case definitions were provided, they were extracted into the evidence table. Case fatality rate (CFR) was extracted as reported. When not explicitly stated, CFR was calculated as deaths divided by cases for that source and reporting period, provided both numerators and denominators were available. When multiple sources reported overlapping national counts for the same reporting period, NCDC situation reports were treated as the primary reference for national totals to reduce double counting. WHO and partner reports were used mainly for triangulation and contextual information (2,4).

Study selection and screening process:

The study selection process followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (22). All retrieved records were deduplicated prior to screening. Titles and abstracts were screened first, followed by full-text assessment against eligibility criteria. Two reviewers (NM and OIA) screened records independently. Discrepancies were resolved by discussion, and where consensus was not reached, a third author (BA) adjudicated. The study selection process is summarized in the PRISMA-ScR flow diagram (Fig 1).

Surveillance dataset for state-by-year analysis:

For each calendar year from 2017 to 2024, state-level case distributions were extracted from the last available NCDC cholera situation report published within that year. Where a year did not have a report close to epidemiological week 52, the most recent sitrep available for that year was used. Counts are cumulative suspected cases as at the epidemiological week covered by the sitrep and do not necessarily represent full-year totals. Percent contributions were calculated within each year using the year total shown in Table 1 as the denominator (4).

Data extraction:

Data were extracted using a standardized form developed a priori and piloted on a sub set of records. Extracted variables were grouped into epidemiological and microbiological or genomic domains. Epidemiological variables included year and, where available, epidemiological week or month; State and LGA; suspected and confirmed cases; deaths; CFR; outbreak duration markers (start and end dates when reported); setting descriptors (community, healthcare facility, IDP setting); and contextual drivers reported by sources (flooding, displacement, WASH disruptions, insecurity, population density, or access constraints).

Microbiological and genomic variables included specimen source (clinical or environmental), isolate counts, confirmation method (culture, PCR, RDT with confirmatory testing where stated), serogroup and biotype descriptors, virulence determinants when reported, antimicrobial susceptibility results and method used (disc diffusion, MIC, automated systems where stated), interpretive standards (CLSI, EUCAST, or not stated), resistance genes or mechanisms (including mobile genetic elements

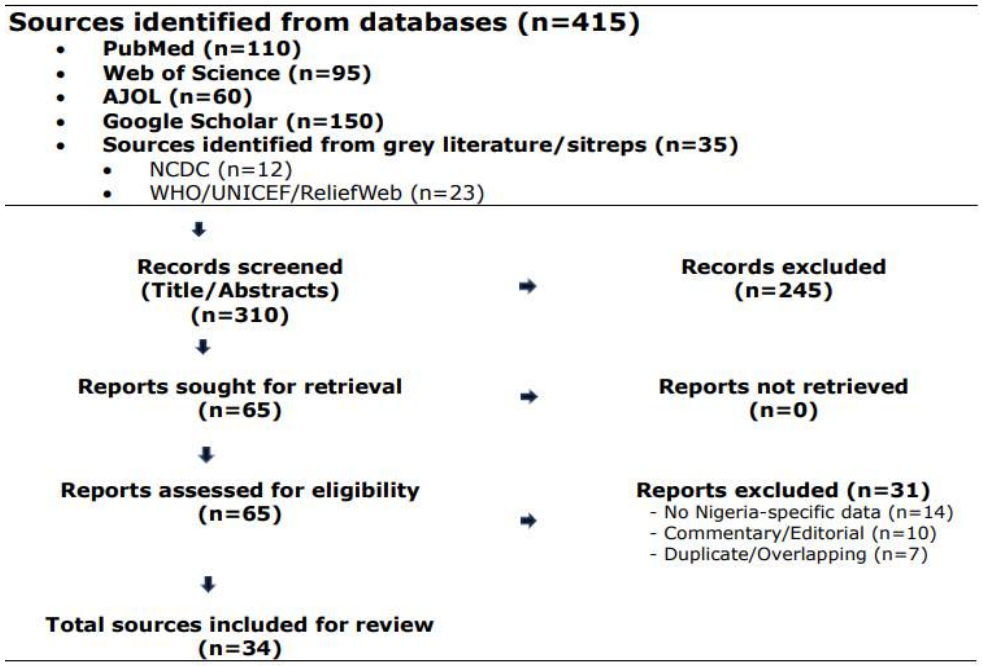

Fig 1: PRISMA-SCR flow diagram

such as ICEs), and reported genomic lineage or clade assignments with the analytical approach described by the original authors. Where multiple publications described overlapping isolate collections or outbreak episodes, we retained the most complete dataset as the primary record and used the others to supplement missing fields.

Quality appraisal and credibility assessment:

Given heterogeneity in evidence types, we applied a structured credibility assessment rather than a single quantitative score. For surveillance and outbreak reports, we assessed clarity of case definitions, reporting completeness, and internal consistency across reporting periods. For microbiology and AMR studies, we assessed clarity of laboratory methods, antimicrobial susceptibility testing methodology, and interpretive criteria. For genomic studies, we assessed transparency of sequencing and analysis methods, including whether lineage assignment and resistance mechanism inference were supported by described pipelines and adequate metadata. Lower-credibility sources were not automatically excluded, but their findings were interpreted cautiously and were not used as the sole basis for key conclusions.

Data synthesis and analysis:

We used narrative and descriptive synthesis. Epidemiological data were summarized by year and state to identify recurrent hotspots and major surge periods. Outbreak timelines were descriptively compared with documented environmental and humanitarian events such as flooding and displacement. Microbiological and genomic findings were synthesized by serogroup, lineage, and resistance mechanism categories. No formal statistical hypothesis testing or meta-analysis was performed. Quantitative results presented are descriptive counts, proportions, and simple derived measures such as CFR when not reported.

Results:

A total of 34 sources were included in the final synthesis. This comprised 21 peer-reviewed primary research articles (covering microbiology, genomics, and epidemiology), 7 NCDC national situation reports (2017–2024), and 6 humanitarian or strategic policy documents providing contextual WASH data. The distribution of cumulative suspected cases across the study period is summarized in Fig 2.

Epidemiology: temporal trends and reporting spread (2017 to 2024):

Across 2017 to 2024, Nigeria reported recurrent cholera surges with substantial year-to-year variation in magnitude and geographic spread (Table 1) (3,4,5). Surveillance analyses covering 2020 to 2021 indicate that cholera affected multiple states with heterogeneous out comes, including variation in reported mortality across settings and time periods (6). Broadly,

national reporting over the review period reflects recurring multi-state outbreaks rather than isolated events, with the number of affected States varying across years (5).

Where demographic distributions were reported in national analyses, children and adolescents accounted for a substantial proportion of cases across multiple outbreak periods, and sex distribution was broadly balanced with modest year-specific differences (3). These demographic patterns are consistent with cholera transmission in contexts where exposure risks are shaped by household water sources, sanitation access, and community-level WASH conditions (23,24).

Geographic clustering and shifting hotspots:

State-level distributions derived from national situation reports indicate repeated high caseloads in northern states across several outbreak years, though the specific States contributing the largest share varied significantly (Table 2). Between 2018 and 2022, the reported burden was frequently concentrated in the North. For instance, in 2018 (week 43), Bauchi, Zamfara, and Katsina together accounted for nearly 60% of the 42,446 suspected cases reported by that period. Similarly, the massive surge in 2021 (week 52), the largest in the study period with 94,886 cases, was driven primarily by Bauchi (20.6%), Jigawa (16.0%), and Kano (12.8%) (4).

However, the geographic concentration shifted notably in 2024. As of week 39, Lagos contributed the largest share (51.2%) of the 9,110 cases reported, marking a significant increase in burden within a densely populated southern urban settlement compared to previous years. This shift contrasts with 2022 (week 52), where Borno alone accounted for 56.1% of the national burden. These state-by-year distributions describe where the reported burden was concentrated at the time of reporting and allow for comparison of relative state contributions, though they do not provide direct year-to-year incidence comparisons due to differing reporting weeks.

Table 1: State distribution of reported suspected cholera cases in Nigeria (2017 to 2024)

Year (last sitrep week)	State	Cumulative suspected cases, n	Share of yearly total, %
2017 (week 36)	Kwara	1,629	31.0
2017 (week 36)	Borno	1,380	26.3
2017 (week 36)	Kebbi	1,263	24.0
2017 (week 36)	Zamfara	430	8.2
2017 (week 36)	Kano	362	6.9
2017 (week 36)	Lagos	70	1.3
2017 (week 36)	Kaduna	39	0.7
2017 (week 36)	Gombe	35	0.7
2017 (week 36)	Jigawa	17	0.3
2017 (week 36)	Oyo	13	0.2
2017 (week 36)	Ebonyi	9	0.2
2017 (week 36)	Ondo	7	0.1
2017 (week 36)	Total	5,254	100.0
2018 (week 43)	Bauchi	9,405	22.6
2018 (week 43)	Zamfara	8,311	20.0
2018 (week 43)	Katsina	7,358	17.7
2018 (week 43)	Borno	6,439	15.5
2018 (week 43)	Adamawa	2,700	6.5
2018 (week 43)	Yobe	2,133	5.1
2018 (week 43)	Kano	1,905	4.6
2018 (week 43)	Sokoto	1,609	3.9
2018 (week 43)	Niger	584	1.4
2018 (week 43)	Gombe	432	1.0
2018 (week 43)	Kaduna	401	1.0
2018 (week 43)	Plateau	347	0.8
2018 (week 43)	Total	41,624	100.0
2019 (week 43)	Adamawa	810	51.2
2019 (week 43)	Bayelsa	526	33.2
2019 (week 43)	Katsina	131	8.3
2019 (week 43)	Kano	58	3.7
2019 (week 43)	Plateau	48	3.0
2019 (week 43)	Ebonyi	9	0.6
2019 (week 43)	Delta	1	0.1
2019 (week 43)	Total	1,583	100.0
2021 (week 52)	Bauchi	19,558	20.6
2021 (week 52)	Jigawa	15,141	16.0
2021 (week 52)	Kano	12,116	12.8
2021 (week 52)	Zamfara	11,931	12.6
2021 (week 52)	Katsina	9,209	9.7
2021 (week 52)	Sokoto	8,477	8.9
2021 (week 52)	Kebbi	5,959	6.3

2021 (week 52)	Borno	5,672	6.0
2021 (week 52)	Yobe	4,002	4.2
2021 (week 52)	Niger	2,821	3.0
2021 (week 52)	Total	94,886	100.0
2022 (week 52)	Borno	12,465	56.1
2022 (week 52)	Yobe	1,888	8.5
2022 (week 52)	Katsina	1,639	7.4
2022 (week 52)	Gombe	1,407	6.3
2022 (week 52)	Taraba	1,153	5.2
2022 (week 52)	Kano	1,131	5.1
2022 (week 52)	Cross River	802	3.6
2022 (week 52)	Bauchi	653	2.9
2022 (week 52)	Zamfara	652	2.9
2022 (week 52)	Jigawa	417	1.9
2022 (week 52)	Total	22,207	100.0
2023 (week 52)	Zamfara	914	28.9
2023 (week 52)	Cross River	718	22.7
2023 (week 52)	Katsina	343	10.8
2023 (week 52)	Bayelsa	319	10.1
2023 (week 52)	Ogun	295	9.3
2023 (week 52)	Ebonyi	236	7.5
2023 (week 52)	Niger	195	6.2
2023 (week 52)	Jigawa	84	2.7
2023 (week 52)	Adamawa	62	2.0
2023 (week 52)	Total	3,166	100.0
2024 (week 39)	Lagos	4,667	51.2
2024 (week 39)	Jigawa	870	9.5
2024 (week 39)	Kano	809	8.9
2024 (week 39)	Borno	588	6.5
2024 (week 39)	Katsina	559	6.1
2024 (week 39)	Adamawa	553	6.1
2024 (week 39)	Bayelsa	513	5.6
2024 (week 39)	Bauchi	307	3.4
2024 (week 39)	Ebonyi	244	2.7
2024 (week 39)	Total	9,110	100.0

Source: Nigeria Center for Disease Control and Prevention (NCDC) sitrep for each year
Note: Counts are cumulative suspected cholera cases reported in NCDC cholera situation reports as at the stated epidemiological week.
Percentages are calculated within each year block using the year total shown in the table.
2020 sitrep was not available.

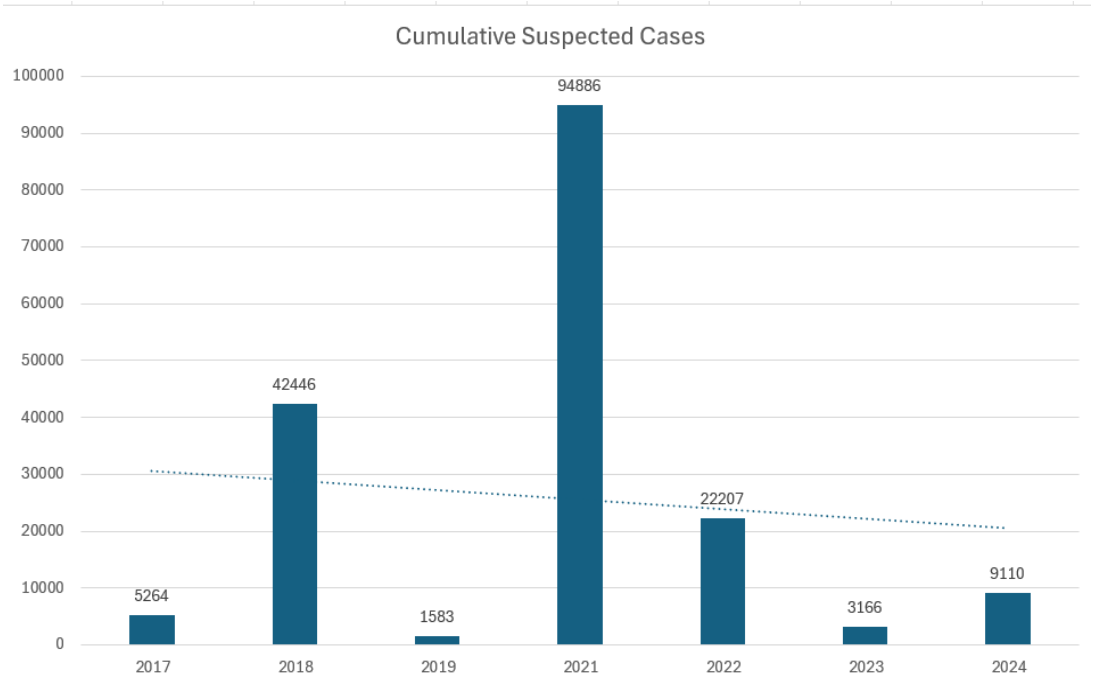

Fig 2: Cumulative cases of suspected cholera cases in Nigeria (2017-2024)

Table 2: Top contributing States by cumulative suspected cholera cases in Nigeria (2017–2024)

Year (Sitrep Week)	Total Cases (N)	Top 3 States	Cases (n)	Share (%)
2024 (week 39)	9,110	Lagos; Jigawa; Kano	4,667; 870; 809	51.2; 9.5; 8.9
2023 (week 52)	3,166	Zamfara; Cross River; Katsina	914; 718; 343	28.9; 22.7; 10.8
2022 (week 52)	22,207	Borno; Yobe; Katsina	12,465; 1,888; 1,639	56.1; 8.5; 7.4
2021 (week 52)	94,886	Bauchi; Jigawa; Kano	19,558; 15,141; 12,116	20.6; 16.0; 12.8
2020	—	Data Unavailable	—	—
2019 (week 43)	1,583	Adamawa; Bayelsa; Katsina	810; 526; 131	51.2; 33.2; 8.3
2018 (week 43)	42,446	Bauchi; Zamfara; Katsina	9,405; 8,311; 7,358	22.2; 19.6; 17.3
2017 (week 36)	5,264	Kwara; Borno; Kebbi	1,629; 1,380; 1,263	31.0; 26.2; 24.0

Note: Total annual cases represent cumulative suspected cases of cholera as of the reported epidemiological week.
Data for 2020 was not captured in the standardized format used for this analysis

Contextual settings described in surveillance and partner reporting:

Across the review period, multiple sources link cholera risk in Nigeria to overlapping contextual drivers, including flooding and displacement in some settings and sanitation constraints in rapidly growing urban areas (6,8,19, 20). The 2022 flooding emergency is repeatedly cited as a major WASH-disrupting event with implications for diarrhoeal disease risks, including cholera (25). In conflict-affected areas, particularly in the Northeast, reports describe constraints on access to safe water and healthcare, with implications for outbreak response capacity in displaced populations (6,26).

In urban settings, literature describing sanitation conditions in informal settlements in Lagos highlights plausible transmission risks where sanitation access is limited and water sources may be contaminated (27,28). These contextual descriptions provide interpretive background but should not be treated as causal inference for specific year patterns without linked analytic designs.

Microbiological and genomic evidence from Nigeria: serogroups, lineages, and AMR patterns:

Predominant serogroups and biotype descriptors reported in Nigeria:

Across published outbreak and laboratory studies that included Nigerian isolates, toxigenic *V. cholerae* O1 has been the dominant serogroup reported in association with cholera outbreaks, most commonly described within the El Tor biotype framework (9,10,18). Studies reporting characterization of isolates from Nigeria describe circulation of El Tor strains and variants with mixed classical and El Tor molecular features, consistent with descriptions of atypical El Tor in West Africa and other cholera-affected regions (9,10).

In addition to O1, non-O1/non-O139 *V. cholerae* strains have been reported from Nigeria in association with diarrhoeal illness or outbreak investigations, although these reports are less frequent than O1-based outbreak reports and are not necessarily captured by routine O1-focused rapid test algorithms (9,12).

Atypical El Tor variants and virulence marker patterns:

Molecular analyses of Nigerian O1 isolates have identified variants described by study authors as atypical El Tor, including strains carrying cholera toxin B subunit alleles associated with classical-like toxin genotypes within an El Tor background (9,10). These findings are relevant because they indicate that Nigerian outbreaks have involved seventh-pandemic lineages with evidence of genetic exchange or replacement events in virulence-associated loci, consistent with patterns described across Africa during the seventh pandemic (11,12). Across studies, the interpretability of virulence marker reporting depends on the methods used (culture confirmation, PCR targets, sequencing), and not all outbreak investigations report comparable marker panels (11,18).

Non-O1/non-O139 isolates and implications for confirmation pathways:

Reports that include non-O1/non-O139 *V. cholerae* isolates from Nigeria emphasize that these strains may be associated with diarrhoeal illness despite lacking the classic O1/O139 serogroup designation used for many cholera surveillance definitions (1). Where such strains are present, outbreak confirmation systems that rely primarily on O1/O139 antigen-based rapid tests may fail to detect them, creating potential under-ascertainment of *V. cholerae* in diarrhoea outbreaks that are clinically and epidemiologically compatible with cholera-like illness (9,12). The practical implication for Nigeria is that surveillance-confirmation pathways benefit from integrating culture and/or nucleic acid detection workflows that can identify *V. cholerae* beyond O1/O139, especially during outbreaks where

rapid tests are negative but clinical suspicion high (3,7).

Antimicrobial susceptibility patterns reported in Nigerian isolate series:

Across isolate series reported from Nigeria, antimicrobial resistance patterns commonly include resistance to older, widely used agents, particularly trimethoprim-sulfamethoxazole and related folate pathway inhibitors, with additional reports of reduced susceptibility to quinolones and other agents depending on outbreak and study period (9,10,13). The systematic review evidence from sub-Saharan Africa supports that resistance in *V. cholerae* is heterogeneous across settings and time, and that variation in testing methods and interpretive criteria limits direct comparability of resistance prevalence estimates across studies (8). Nigeria-specific reports similarly differ in antimicrobial panels tested, susceptibility testing approach, and reporting standards, which constrains attempts to infer national resistance trends from the published literature alone (10,29).

Where resistance mechanisms were explored, studies describe roles for mobile genetic elements, including integrative conjugative elements (ICEs), and for target-site mutations relevant to quinolone susceptibility, consistent with established mechanisms in seventh-pandemic *V. cholerae* (1,2,3). However, because many outbreak reports do not include isolate-level antimicrobial susceptibility testing, the degree to which published resistance profiles represent contemporaneous national outbreak strains is uncertain (3,7).

Genomic lineage assignments and regional clustering including Nigerian isolates:

Genomic studies that include Nigerian isolates place them within seventh-pandemic El Tor diversity and identify regional clustering patterns across Africa and West Africa. The Africa-wide genomic reconstruction of seventh-pandemic spread identifies multiple introductions and lineages circulating on the continent, providing context for Nigerian isolates within broader transmission histories (11). West Africa focused sequencing work reports persistence of regional lineages over time and includes Nigerian isolates within lineage groupings described by study authors, including T12, with continuity into AFR12 designations in more recent multi-country analyses (4,9). These studies support that Nigerian outbreak strains are part of regional transmission systems rather than isolated national lineages, although the density of Nigeria-only sequencing across all outbreak years is limited, constraining fine-scale inference about within-country transmission routes and state-to-state connectivity in some years (12, 14).

Laboratory confirmation gaps as a cross-cutting limitation for microbiological interpretation:

Across surveillance reports and published analyses, limited laboratory confirmation is repeatedly identified as a constraint for characterizing outbreak strains and tracking AMR during response (3,7). Over-reliance on rapid diagnostic tests without systematic confirmatory culture and susceptibility testing can delay detection of resistance patterns and limit the ability to link epidemiological hotspots to pathogen characteristics. This limitation also affects interpretation of whether observed epidemiological shifts reflect true changes in pathogen circulation, changes in exposure and vulnerability, or changes in testing and reporting completeness (3,7).

Public health responses reported (OCV, WASH and outbreak operations)

Vaccination activities and constraints (oral cholera vaccine):

Across the reviewed sources, oral cholera vaccine (OCV) is consistently described as a key outbreak control and prevention tool, but with operational constraints that affect coverage and timing in Nigeria. Reports and analyses cite limited vaccine availability and distribution challenges as barriers to achieving coverage levels considered programmatically useful in high-risk settings, particularly during periods of global OCV supply constraint (30,31). Implementation barriers are described as more pronounced in insecure settings, where access limitations and displacement complicate campaign logistics (16,32). Where campaigns were implemented, sources describe temporal associations between targeted vaccination activities and subsequent reductions in reported cases, although these accounts are typically descriptive and not designed as causal evaluations (4,16).

WASH interventions documented during outbreaks:

Water, sanitation and hygiene interventions reported during outbreak periods include emergency water point chlorination, hygiene kit distribution, and WASH service strengthening in high-risk communities. UNICEF and partner reports describe chlorination and hygiene support activities implemented in outbreak-prone areas, including north-west settings, alongside broader WASH response efforts in the northeast through collaboration with international and national partners (21,33,34). Humanitarian updates and analytical reviews also link major flooding events to WASH disruption and subsequent out-

break risk, and they describe scale-up of emergency response activities during flooding-associated crises (19,20,25). These sources emphasize operational challenges in sustaining safe water access over time, particularly when infrastructure damage, population displacement, and monitoring constraints limit consistent water quality control (33,35).

Outbreak operations, insecurity, and access constraints:

Several sources describe how conflict and insecurity affect cholera response operations in Nigeria, particularly in the Northeast. These constraints include disrupted water and sanitation infrastructure, limited access for responders, and fragmentation of routine healthcare services in displaced populations (6,8). Situation reporting from affected areas describes concurrent challenges from flooding and health system strain during outbreak response periods, with implications for timely detection, case management, and implementation of community-level interventions (26,32). These contextual reports are consistent with broader analyses linking conflict and cholera in Nigeria and emphasizing the compounding effects of displacement and service disruption on outbreak dynamics (6,15).

Diagnostic and laboratory capacity in response reporting:

Across surveillance analyses and policy-focused reviews, diagnostic gaps are repeatedly described as a limiting factor for outbreak confirmation and for microbiological characterization during response. Sources describe reliance on suspected case reporting and variable laboratory confirmation, with implications for differentiating cholera from other acute watery diarrhoea causes and for linking epidemiological patterns to pathogen characteristics such as lineage and antimicrobial resistance (6,7). This limitation is presented as operational rather than purely technical, reflecting specimen referral constraints, uneven culture capacity, and inconsistent linkage between field detection and laboratory confirmation workflows.

National strategic planning for cholera control:

The reviewed sources also point to an increased focus on national-level planning that aims to integrate surveillance, laboratory strengthening, WASH, and vaccination strategies within defined implementation frameworks. Recent analyses discuss enabling factors and barriers for cholera intervention implementation, emphasizing governance, logistics, and community engagement as determinants of feasibility (7,16). These sources describe strategic intent to align prevention and response activities with global cholera control approaches, while acknowledging that execution is shaped by financing, operational capacity, and the realities of insecurity and climate-related shocks in high-risk settings (7,15).

Discussion:

This integrative review synthesizes surveillance reporting (2017 to 2024) alongside microbiological and genomic evidence relevant to Nigeria and supports five main findings. First, cholera in Nigeria during 2017 to 2024 was characterized by recurrent surges with shifting geographic concentration. State-by-year distributions derived from NCDC sitreps show that reported burden frequently clustered in northern states across multiple years, while later reporting also highlighted large burdens in densely populated urban settings, including Lagos in 2024 within the extracted distribution (4). National surveillance analyses similarly describe multi-state outbreaks with heterogeneous outcomes and recurring transmission across years rather than isolated, self-limited events (3,5).

Second, multiple sources consistently describe contextual conditions that plausibly amplify transmission and complicate response, including conflict-related displacement and access constraints in parts of the North, and flooding-linked disruption of water and sanitation services in several regions (6,8,25,32). In urban informal settlements, published work describing sanitation constraints in Lagos provides a plausible environmental and social basis for sustained diarrhoeal disease transmission risks, although these sources are not designed to attribute causality to specific outbreak years contaminated (27,28).

Third, the microbiological literature indicates that outbreaks in Nigeria are predominantly associated with seventh-pandemic *V. cholerae* O1 El Tor, including atypical El Tor variants described in Nigerian isolate series (9,10, 18). These findings align with broader African genomic reconstructions that place West African outbreaks within seventh-pandemic spread and persistence patterns (11). Fourth, antimicrobial resistance patterns reported from Nigerian isolates commonly include resistance to agents such as trimethoprim-sulfamethoxazole, with reports of reduced quinolone susceptibility in some datasets and mechanistic evidence implicating mobile genetic elements and target-site changes, consistent with established resistance mechanisms in seventh-pandemic *V. cholerae* (9,10,11,13). However, the Nigeria-specific evi-

dence base is methodologically heterogeneous, and isolate series are often outbreak- or site-specific, limiting inference about national resistance prevalence or temporal trends (13,29).

Fifth, genomic studies that include Nigerian isolates support regional clustering and connectivity within West African seventh-pandemic transmission systems. West Africa-focused sequencing and more recent multi-country genomic analyses include Nigerian isolates within lineage groupings described by study authors such as T12 and AFR12, reinforcing the interpretation that Nigeria's outbreak strains are part of regional transmission networks (4,9). The main limitation is that Nigeria-only sequencing density remains insufficient across all years to consistently resolve within-country transmission pathways or to link state-by-state epidemiology to fine-scale phylogenetic structure (12, 14).

Nigeria's surveillance patterns and the wider contextual evidence suggest that cholera burden is shaped by the intersection of environmental shocks, population movement, and structural limitations in safe water and sanitation. Analyses linking conflict and cholera in Nigeria emphasize how insecurity and displacement can increase exposure risks through crowded living conditions and disrupted WASH infrastructure, while simultaneously limiting access for responders and reducing health system capacity for prompt case management and outbreak control (6,8). In these settings, even when suspected case reporting is strong, laboratory confirmation and isolate characterization may lag due to specimen referral constraints, limited culture capacity, and operational risk, which in turn reduces the ability to generate real-time microbiological intelligence to guide response (3,7).

Flooding is repeatedly described as a major WASH-disrupting event in Nigeria with implications for diarrhoeal disease outbreaks, including cholera. The 2022 flooding emergency is consistently highlighted in the literature and humanitarian reporting as a large-scale displacement and infrastructure disruption event that plausibly increased exposure through contamination of water sources and interruption of routine sanitation services (19,20,25). Such flood-linked amplification does not negate the importance of endemic persistence and recurrent introductions, but it underscores why outbreak magnitude may vary sharply by year even when underlying vulnerabilities remain similar.

The contribution of urban informal settlements to cholera risk is supported by sanitation literature describing high-risk environmental conditions in parts of Lagos, including limited access to improved toilets and reliance on compromised water sources in some communities (27,35). These conditions are compatible with cholera transmission pathways and may become increasingly relevant as urban populations expand. The implication for cholera control is that surveillance and prevention strategies should not assume that risk is confined to rural or conflict-affected northern settings, even if those settings account for large reported burdens in many years (4,7).

A consistent operational weakness across sources is limited laboratory confirmation relative to suspected case reporting. This has direct consequences. When confirmation is delayed or absent, outbreak response may proceed without clear pathogen characterization, limiting the ability to detect and respond to changes in serogroup distribution, virulence profiles, or antimicrobial susceptibility (3,7). It also complicates rational antibiotic policy for severe cases. Even if antibiotic therapy is not indicated for most mild cholera cases, severe disease and hospitalized patients may require antimicrobial therapy to reduce stool volume and duration, and local susceptibility data are needed to support empiric guidance and stewardship (7,13).

The presence of non-O1/non-O139 *V. cholerae* reports in Nigeria further supports strengthening confirmation pathways beyond O1/O139-focused antigen testing. While O1 remains predominant in cholera outbreaks, non-O1/non-O139 isolates associated with diarrhoeal illness introduce a diagnostic vulnerability if confirmation workflows depend mainly on O1-targeted rapid tests, particularly when clinical suspicion is high but initial antigen testing is negative (9,12). Practical approaches include ensuring culture capacity at reference laboratories, predefined specimen referral routes during outbreaks, and targeted molecular testing panels where feasible to support timely identification and characterization (3,7).

The available Nigeria-specific microbiology indicates that resistance to trimethoprim-sulfamethoxazole and related agents is common in reported isolate series, while reduced quinolone susceptibility and other resistance patterns appear in some datasets, supported by described mechanisms including mobile genetic elements and target-site changes (9,10,13). These findings suggest that empiric antibiotic guidance for severe cholera in Nigeria should not assume susceptibility to older oral agents and should be based on locally generated susceptibility data where available, interpreted with awareness of methodological variability (13,29). They also support the inclusion of antimicrobial stewardship principles in outbreak res ponse planning, especially where inappropriate antibiotic expo-

sure may be common in informal care settings, potentially selecting for resistant strains and complicating management of other bacterial infections (7).

Genomic studies that include Nigerian isolates provide evidence for regional clustering and connectivity within West Africa and support the interpretation that Nigeria's outbreaks are linked to broader transmission systems rather than confined national lineages (11,12,14). The operational value of genomics for Nigeria depends on shortening the distance between sequencing outputs and outbreak decision-making. For many outbreak years, sequencing evidence remains retrospective or dependent on external collaborations, limiting real-time utility (12,14). Priority steps include establishing minimum metadata standards for isolate submission, ensuring integration between surveillance reporting and laboratory information systems, and defining practical sequencing triggers, such as a defined number of isolates per outbreak cluster or per high-burden state to support lineage and resistance mechanism tracking (7).

Sources consistently describe OCV as an important tool but emphasize supply and operational constraints, including global shortages and access barriers in insecure areas (10,31, 32). Where implemented, vaccination campaigns are described as potentially impactful, but most available reporting is descriptive rather than evaluative (4). WASH interventions such as water point chlorination and hygiene kit distribution are widely reported, often implemented in emergency response contexts, but sustained safe water provision remains challenging where infrastructure damage, displacement, or monitoring constraints limit continuity (21,35). The practical implication is that cholera control strategies require alignment between immediate emergency WASH measures and longer-term sanitation and water infrastructure improvement, with prioritization of settings repeatedly identified in surveillance reporting (7,15).

This review has important limitations. First, the epidemiological findings rely largely on routine outbreak reporting, which is affected by under-ascertainment, variable case definitions over time and across settings, and uneven access to laboratory confirmation. These issues reduce comparability across states and years and can bias apparent geographic concentration toward locations with stronger reporting systems or better access to care and testing (3,4, 7). Second, the State-by-year distributions in Table 1 were extracted from the last available NCDC situation report within each calendar year. The counts are cumulative as at the epidemiological week covered by that report and may not represent full-year totals. Because the last reporting week differs by year, data in Table 1 are most appropriate for within-year state comparisons rather than direct year-to-year comparisons of incidence or national burden.

Third, the microbiological and antimicrobial resistance evidence is heterogeneous. Studies differ in outbreak context, sampling frames, isolate selection, antimicrobial panels, susceptibility testing methods, and interpretive standards. This limits comparability across studies and prevents reliable pooled estimates of resistance prevalence or inference of true temporal trends (13,29). Fourth, the genomic evidence relevant to Nigeria is uneven over time and often derived from multi-country datasets that include Nigerian isolates rather than continuous Nigeria-only sequencing. While these data support lineage assignment and regional clustering, they limit fine-scale inference about within-country transmission dynamics in some years and settings (12,14).

Finally, grey literature and humanitarian reporting add valuable context on flooding, displacement, and response activities, but these sources vary in methodological detail, outcome definitions, and reporting consistency. They are useful for triangulation and operational interpretation but should be treated cautiously for quantitative inference (19,20,25).

Implications and recommendations:

The evidence in this review points to practical priorities that connect surveillance, laboratory confirmation, antimicrobial stewardship, and prevention. Outbreak response needs dependable confirmation. Rapid specimen referral from treatment sites to culture-capable laboratories should be pre-defined, with minimum confirmation targets per outbreak cluster, routine susceptibility testing on a subset of confirmed isolates, and standardized reporting that links confirmation method, serogroup, and AST results to surveillance line lists. Where RDTs are used widely, confirmatory testing should be built into routine outbreak workflow so results can inform case management and messaging in real time (3,7).

Antibiotic guidance for severe cholera should reflect Nigeria-relevant susceptibility data and be updated periodically. Where local data are limited, guidance should state uncertainty and avoid agents with frequent resistance in reported isolate series. Stewardship should be integrated into outbreak case management because outbreaks often drive inappropriate antibiotic use in communities and informal care settings (7,9,10,13,29).

Genomics should be used for defined operational decisions, not only retrospective description. Priority uses include distinguishing linked outbreaks from multiple introductions, tracking resistance mechanisms and mobile genetic elements, and enabling regional co-ordination through comparable sequence data and metadata. A feasible minimum is a tiered sequencing plan with a standard subset of isolates from high-burden states and major outbreaks each year (7,11,12,14).

Prevention should concentrate on repeatedly high-risk settings, including conflict and displacement contexts and flood-affected communities with WASH disruption, while also addressing urban informal settlements. Packages should combine predictable emergency WASH actions during outbreaks, sustained safe-water strategies where feasible, and targeted OCV when doses are available, adapted- to insecurity and access constraints (6,7,16,25,27). Finally, reporting comparability needs improvement. A minimum national outbreak dataset with consistent case definitions, denominators, confirmation counts, and key response actions would reduce confusion across years and partners and allow clearer linkage between epidemiology and laboratory outputs (3,7).

Conclusion:

Nigeria experienced recurrent cholera surges from 2017 to 2024 with shifting geographic concentration and persistent vulnerability in settings affected by displacement, flooding, and inadequate sanitation. The microbiological and genomic literature indicates that outbreaks are predominantly associated with seventh-pandemic *V. cholerae* O1 El Tor lineages, including atypical El Tor variants, and that AMR determinants reported in Nigerian isolate series have implications for empiric management of severe disease. Genomic studies that include Nigerian isolates support regional clustering within West Africa, but Nigeria-only sequencing density remains insufficient for consistent within-country resolution across all outbreak years.

Strengthening outbreak-linked confirmation, routine susceptibility testing, and feasible sequencing pathways, alongside integrated WASH and targeted vaccination strategies, would improve both response effectiveness and the evidence base needed for sustained cholera control in Nigeria (3,7,12).

Contributions of authors:

BA conceived the study; NM prepared the first draft of the manuscript; NM, OIA, and RE performed data curation and descriptive analysis; and all authors (NM, OIA, IJ, RE, RDG, BA) critically reviewed, revised, and approved the final manuscript.

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Authors declare no conflicts of interest.

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Original Article **Open Access**

Clinical characteristics and outcomes of patients with acute watery diarrhoea admitted to the Cholera Treatment Center of Yekatit-12 Teaching Hospital, Addis Ababa, Ethiopia

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Abstract

Background: Cholera remains a major public health concern in low-income countries where the spread of disease is exacerbated by poor water supply, hygiene, and sanitation. Ethiopia has continued to face recurrent cholera outbreaks despite efforts to prevent and control the disease. The aim of the study was to assess the clinical characteristics, and outcomes among patients with acute watery diarrhea admitted to Cholera Treatment Center (CTC) of Yekatit-12 Teaching Hospital, Addis Ababa, Ethiopia.
Methodology: A hospital-based cross-sectional study was conducted from July 1, 2023 to Feb 30, 2025 on 176 patients with acute watery diarrheal disease admitted to the CTC during two major rainy seasons. Relevant clinical variables including socio-demographic data, co-morbidities, clinical presentations, risk factors, complications, outcomes and laboratory investigation results were obtained. Descriptive statistics were used to summarized the data and associations were tested using logistic regressions with statistical significance set at p value <0.05.
Results: Of the 176 patients, 52.0% were female and 90.0% were ≥15 years old. Vomiting and diarrhea were the predominant presenting symptoms (71.8%); 36.7% and 50.8% of patients had some dehydration and severe dehydration at admission, respectively. Electrolytes disturbance was the leading complication with hypokalemia (45.8%) being the most common, followed by hyponatremia (34.5%), and hypochloremia (23.7%). Acute kidney injury (AKI) occurred in 38.4% of the patients. The risk of AKI among patients >45 years was 5.9 times higher than patients <15 years of age (95% CI: 1.501-23.3, p=0.01) and patients with severe dehydration had 3.96 times higher risk of AKI compared with no dehydration (95% CI: 1.205-13.018, p=0.023). The case fatality rate was 6.25%. The common exposures to the disease included eating outside of home (54.0%), and unsafe water consumption (85.0%).
Conclusion: The cholera outbreak was characterized by moderate to severe dehydration, high burden of electrolyte imbalance, AKI and case fatality rate. Early case detection and community water, sanitation and hygiene (WaSH) interventions are recommended to reduce the disease burden, morbidity and mortality.

Keywords: Acute watery diarrhoea; Cholera; Acute kidney injury; Outcome; Ethiopia

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Caractéristiques cliniques et évolution des patients atteints de diarrhée aqueuse aiguë admis au Centre de Traitement du Choléra de l'Hôpital Universitaire Yekatit-12, Addis-Abeba, Éthiopie

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Résumé:

Contexte: Le choléra demeure un problème majeur de santé publique dans les pays à faible revenu, où la propagation de la maladie est exacerbée par l'insuffisance de l'approvisionnement en eau, de l'hygiène et de l'assainissement. L'Éthiopie continue de faire face à des épidémies de choléra récurrentes malgré les efforts déployés pour prévenir et contrôler la maladie. L'objectif de cette étude était d'évaluer les caractéristiques cliniques et l'évolution des patients atteints de diarrhée aqueuse aiguë admis au Centre de traitement du choléra (CTC) de l'hôpital universitaire Yekatit-12 d'Addis-Abeba, en Éthiopie.
Méthodologie: Une étude transversale en milieu hospitalier a été menée du 1er juillet 2023 au 30 février 2025 auprès de 176 patients atteints de diarrhée aqueuse aiguë admis au CTC au cours de deux saisons des pluies majeures. Les variables cliniques pertinentes, notamment les données sociodémographiques, les comorbidités, les manifestations cliniques, les facteurs de risque, les complications, l'évolution et les résultats des analyses de laboratoire, ont été recueillies. Des statistiques descriptives ont été utilisées pour résumer les données et les associations ont été testées par régression logistique, le seuil de signification statistique étant fixé à $p<0,05$.
Résultats: Parmi les 176 patients, 52,0% étaient des femmes et 90,0% étaient âgés de 15 ans ou plus. Les vomissements et la diarrhée étaient les symptômes les plus fréquents (71,8%). À l'admission, 36,7% des patients présentaient une déshydratation légère et 50,8% une déshydratation sévère. Les troubles électrolytiques constituaient la principale complication, l'hypokaliémie (45,8%) étant la plus fréquente, suivie de l'hyponatrémie (34,5%) et de l'hypochlorémie (23,7%). Une insuffisance rénale aiguë (IRA) est survenue chez 38,4% des patients. Le risque d'IRA était 5,9 fois plus élevé chez les patients de plus de 45 ans que chez ceux de moins de 15 ans (IC à 95 %: 1,501-23,3; $p=0,01$), et ce risque était 3,96 fois plus élevé chez les patients présentant une déshydratation sévère que chez ceux ne présentant pas de déshydratation (IC à 95%: 1,205-13,018; $p=0,023$). Le taux de mortalité était de 6,25%. Les principaux facteurs de risque étaient les repas pris à l'extérieur (54,0%) et la consommation d'eau non potable (85,0%).
Conclusion: L'épidémie de choléra a été caractérisée par une déshydratation modérée à sévère, une forte prévalence de déséquilibres électrolytiques, une insuffisance rénale aiguë et un taux de létalité élevé. Le dépistage précoce des cas et des interventions communautaires en matière d'eau, d'assainissement et d'hygiène (EAH) sont recommandés pour réduire la morbidité et la mortalité liées à la maladie.

Mots-clés: Diarrhée aqueuse aiguë; Choléra; Insuffisance rénale aiguë; Évolution; Éthiopie

Introduction:

Cholera has been a global threat to health security, particularly in sub-Saharan Africa where outbreaks have become frequent, with increased magnitude and fatalities (1,2). It is one of the key indicators of poor socio-economic development and remains a challenge to countries where access to safe drinking water and adequate sanitation cannot be guaranteed (3). The outbreak requires an immediate response including rapid mobilization of resources including Water and Sanitation, and Hygiene (WaSH) to control the spread and prevent fatalities (3, 4).

Cholera is an extremely virulent disease caused by infection of the intestine with Gram-negative bacteria *Vibrio cholerae*. The onset of illness may be sudden, with profuse, painless, watery diarrhea leading to severe and rapidly progressing dehydration, hypovolemic shock and death (5). Without adequate and appropriate rehydration therapy, severe cholera kills more than 50% of affected individuals but <1% if appropriately treated (5,6). Only 20-25% of cholera cases are symptomatic, of which 10-20% can develop severe disease, and the remaining 80% are asymptomatic but still can transmit the infection (7).

The true global burden of cholera is largely unknown as the passively acquired surveillance data under-reports its cases related to poor surveillance systems, poor laboratory diagnostic capacities, and socio-politico-economic disincentives for reporting in many cholera-endemic countries (8,9). Consistent with this finding, the WHO estimated that only 5–10% of the cholera cases occurring annually are officially reported by member countries (10). Studies show that Africa and southern Asia account for about 99% of the cholera burden worldwide, primarily due to poverty, including inadequate sanitation, contaminated drinking water, poor food hygiene, and lack of health care services (11,12).

Ethiopia, in its long history, has endured several outbreaks of acute watery diarrhea (AWD) due to cholera. Over the recent 9 years alone, there has been reported a total of 99,945 cases, with case fatality rate (CFR) reaching up to 3.13% (13,14). Due to extensive magnitude of the problem, the Ethiopian government established multi-sectoral cholera elimination plan (NCP) and updated its cholera surveillance and outbreak response guidelines (16). The guidelines have incorporated a collaborative effort to monitor and manage cholera outbreaks across borders, refined the case definitions of cholera, and also integrated oral cholera vaccine (OCV) as a crucial component of cholera control and prevention strategy (13,16).

Despite the extensive efforts, in the last

one decade alone, multiple cholera outbreaks have been reported in different regions of the country (9,13,17,18,19). The recent outbreak started in August 2022 in Oromia region of Ethiopia and later spread to other areas of the country, including Addis Ababa, and has been a growing concern (15). Since almost all of the outcome data in Ethiopia were based on the retrospective studies or second-hand data which were less likely to capture the real time account regarding clinical presentations, degree of dehydrations, and complications of cholera, including the mortality rate. Therefore, our facility-based study was aimed to generate first-hand account about the clinical characteristics, risk factors, complications, and treatment outcomes among cholera suspected patients with AWD admitted to Cholera Treatment Center (CTC) of Yekatit-12 Hospital Medical College.

Materials and method:

Study setting:

The study was conducted in the medical isolation ward currently dedicated for Cholera Treatment Center (CTC) after the recent outbreaks of acute watery diarrhea (AWD) at the Yekatit-12 Hospital Medical College from July 1, 2023 to February 30, 2025. The outbreaks reported were mainly during the 2023 and 2024 Ethiopian rainy seasons.

Study design:

A hospital-based cross-sectional study was conducted involving all AWD patients admitted to the CTC of Yekatit-12 Hospital Medical College after fulfilling the case definition of cholera or tested positive for cholera during the outbreak.

Ethical approval and consent for participant:

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical clearance was obtained from institutional review board of Yekatit-12 Hospital medical College. Written informed consent was obtained from the study participants before the commencement of data collection.

Operational definition:

The case definition of cholera in a region where cholera outbreak was declared, is any person aged 2 years or more presenting with or dying from acute watery diarrhea (4). Acute kidney injury (AKI) was diagnosed when there is an absolute increase in serum creatinine (sCr) by ≥0.3 mg/dL (26.5 µmol/L) within 48 hours or by 1.5 times increase in sCr from baseline within 7 days, or a urine volume of <0.5 ml/kg/h for at least 6 hours (20). Hypokalemia was defined when the serum potassium level <3.5 meq/ L (21). Hypochloremia was defined as serum chloride level < 96 meq/L (21). Hyponatremia was defined when serum sodium is < 135 mEq/L (21).

Severity of dehydration in a patient with AWD was assessed by using key clinical indicators; (i) No Dehydration (DHN) if no characteristic signs, (ii) Some Dehydration if ≥2 of any characteristic signs including ≥1 major sign of some DHN, and (iii) Severe Dehydration if ≥2 of any characteristic signs including ≥1 major sign of severe DHN as shown in Table 1 (4).

Table 1: Classification of the severity of dehydration in patient with acute watery diarrhea in Addis Ababa, Ethiopia

Status	No Dehydration	Some Dehydration	Severe Dehydration
Check for pulse	Present, Rapid	Weak, Thready	No pulse
Patient condition	Well, Alert	Restless, Irritable‡	Lethargic or unconscious‡
Eye sunken	No	Yes (sunken)	Yes (very sunken and dry)
Mouth and tongue	Moist	Dry	Very dry
Thirst*	Drinks normally	Thirsty, drinks eagerly‡	Drinks poorly or not able to drink‡
Skin pinch	Goes back quickly	Goes back slowly‡	Goes back very slowly (>2 seconds)‡
Decide	The patient has no signs of dehydration	If the patient has ≥ 2 signs, including ≥1 major sign, there is some dehydration	If the patient has ≥ 2signs, including ≥ 1 major sign, there is severe dehydration
Treat	Maintain hydration	Oral rehydration solution	IV + ORS + Antibiotic
	PLAN A	PLAN B	PLAN C

Source: FDEMoH Guideline for Cholera Surveillance and Outbreak Response, 2022.
*Patient should be offered fluid to observe for this sign,
**Abdominal skin has to be pinched and released to observe for this sign,
‡Major signs

Data collection:

We utilized KoboToolbox for data collection. In order to ensure the accuracy, completeness, and comparability of data, two senior medical residents were trained to complete data collection format. Relevant clinical data including socio-demographic data, co-morbidities, clinical presentations, risk factors, complications, and outcomes were obtained. Necessary laboratory investigations including renal function test, serum electrolyte, and stool examination were done in Yekatit-12 Hospital.

Data processing and statistical analysis:

After checking for completeness, data were coded, entered, and analyzed using SPSS version 25 software package. Descriptive statistics were used to calculate rates. Multiple logistic regression analysis was used to check for association of dependent variable with independent variables. A p value < 0.05 was taken as statistically significant.

Results:

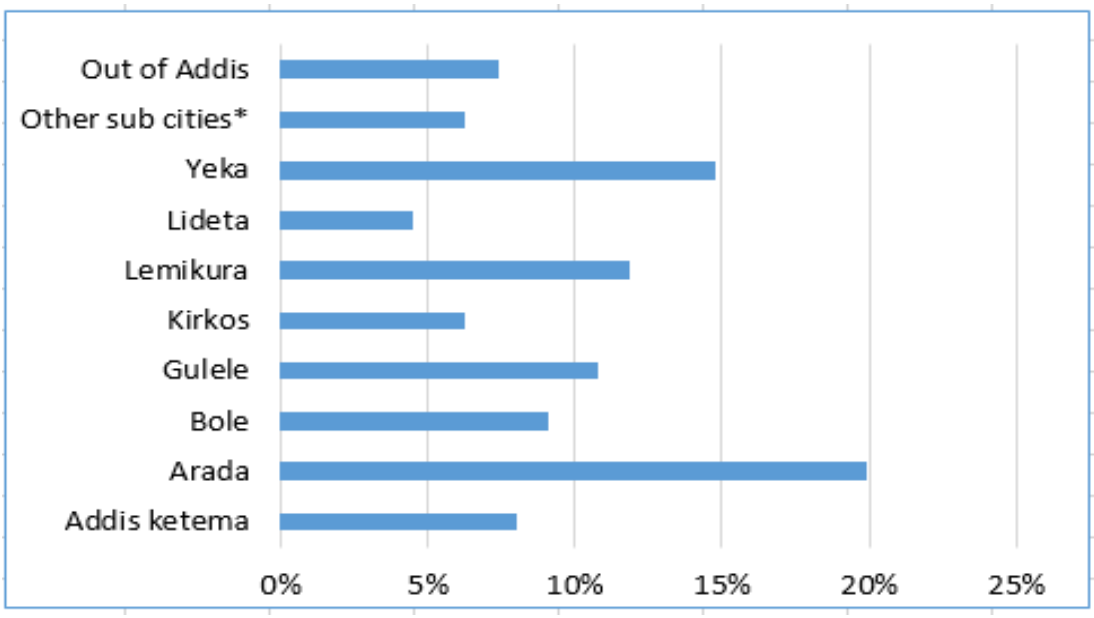
Demographic characteristics of patients with suspected cholera:

From all 176 acute watery diarrheal patients suspected of cholera and admitted to the Cholera treatment center (CTC), females accounted for about 52.0% (n=91), over 45.0% had no formal education or educated only up to elementary school, and about one third of the patients had monthly income of less than 5000 Ethiopian Birr (Table 2).

About 93.0% of patients were from Addis Ababa, and the remaining 7.3% were referred from outside Addis Ababa. The suspected cases of cholera were from all the 11 sub-cities of Addis Ababa, with over 46.0% from Arada, Yeka, and Lemikura sub-cities in descending order (Fig 1). Only 16.7% of adults were living alone, and about 40.0% of patients were living in a family size of 5 and above.

Table 2: Demographic characteristics of patients with acute watery diarrhea admitted to the Cholera Treatment Center (CTC), Addis Ababa, Ethiopia

Characteristics		**Number**	**Percentage (%)**
Age group (years)	<15	19	10.8
	15-45	79	44.9
	>45	78	44.3
Sex	Male	85	48.3
	Female	91	51.7
Marital status	Single	60	34.1
	Married	85	48.3
	Widowed and divorced	31	17.6
Level of education	No formal education	35	19.9
	Preschool children	15	8.5
	Elementary	45	25.6
	High school	65	36.9
	College and above	16	9.1
Religion	Orthodox Christianity	135	76.9
	Protestant Christianity	29	16.5
	Muslim	9	5.1
Residence	Addis Ababa	161	91.5
	Outside Addis Ababa	15	8.5
Ethnicity	Amhara	78	44.3
	Oromo	36	20.5
	South Ethiopia	51	29
	Others	9	5.1
Income (Et Birr)	<1000	11	6.3
	1000-4999	43	24.4
	5000-10000	49	27.8
	>10000	4	2.3



*Kolfe (n=6, 3.4%), Nifas Silk (n=3, 1.7%), and Akaki (n=2, 1.1%)

Fig 1: Distribution of acute watery diarrhea patients suspected of cholera in Sub-cities of Addis Ababa, Ethiopia

Clinical characteristics and management of patients with suspected cholera:

In the study setting, the outbreak of cholera was declared (1,17,18,25) and substantiated by microbiological evidence from the stool of patients with AWD. Subsequent assessment of cholera among over 90.0% of patients presented with AWD was made according the case definitions as there was lack of diagnostic facilities. The major presenting symptoms among the AWD patients admitted were watery diarrhea in 27.7% of cases, and the remaining over 90.0% of patients had both watery diarrhea and vomiting of ingested matter. About 44.0% of patients had 1 day and/or less duration of illness at presentation, whereas about a quarter of patients had more than 3 days of illness at presentation. About half of the patients had an admission diagnosis of AWD with some DHN, and 36.7% of patients had AWD with severe DHN (Table 3).

Since early diagnosis and rapid management of dehydration are crucial among AWD patients suspected of cholera, all the patients admitted to the CTC had received generous fluid therapy. Oral rehydration salt (ORS) was given to all patients with No Dehydration (AWD with No DHN), and patients with some dehydration (AWD with Some DHN). ORS was also given for patients with severe dehydration (AWD with Severe DHN) whenever they can tolerate oral therapy. About 65% of patients received intravenous (Ringer's Lactate) fluid therapy; all patients with severe dehydration (AWD with Severe DHN), and 49% of patients with some dehydrations (AWD with Some DHN) were given IV fluid up on the discretion of the treating physicians while monitoring their clinical condition.

About 88% of patients with severe DHN, and 50% with some DHN received antibiotics, mostly stat oral doxycycline of 300mg or IV antibiotics at the consideration of concomitant physician diagnosis of infections including sepsis, gastrointestinal infection, sepsis or chest infection. A third of patients developed hyponatremia, a quarter hypochloremia, and one-third of patients were treated with KCl for moderate or severe hypokalemia. About 75% of the patients had a hospital stay of at least 5 days (Table 3).

Factors associated with renal complications in patients with suspected cholera:

The mortality rate among patients with AWD suspected of cholera and admitted to the CTC was 6.25% (Table 3). All patients who died in the CTC had acute kidney injury (AKI) and 90.1% of them had an admission diagnosis of AWD with severe DHN or shock. Over 38% of AWD patients admitted to CTC developed acute kidney injury (AKI).

The risk of developing AKI was significantly increased with increased age, electrolyte disturbance, and level of dehydration. The risk of AKI among older patients aged above 45 years of age was 5.9 times higher than younger patients aged less than 15 years (95% CI: 1.501-23.3, p=0.01). Patients staying longer than 3 days in the hospital had 3.8 times higher

risk of AKI (95% CI: 1.8-8.05, p=0.001), and patients with severe dehydration had 3.96 times higher risk of AKI compared with patients no dehydrations (95% CI:1.205-13.018, p=0.023) (Table 4). Although the burden of AKI was higher among females, and patients with co-morbidities, the differences were not statistically significant.

Table 3: Clinical characteristics of patients with acute watery diarrhoea admitted to the Cholera Treatment Center in Addis Ababa, Ethiopia

Clinical characteristics	**Parameters**	**Frequency**	**Percent**
Confirmed cholera	Confirmed cholera by stool examination	13	7.3
	Clinical diagnosis of cholera	164	92.7
Major symptoms	Watery diarrhea	49	27.7
	Watery diarrhea and vomiting	127	71.8
Duration of illness at presentation	<2 days	54	43.9
	2-3 days	40	32.5
	>3 days	28	22.8
Admission diagnosis with respect to dehydration	AWD with No Dehydration	21	11.9
	AWD with Some Dehydration	90	50.8
	AWD with Severe Dehydration	65	36.7
Treatment	Oral Rehydration Salt (ORS)	176	100
	Ringer's Lactate	115	65.3
	Antibiotics	103	58.5
	Potassium Chloride (KCL)	55	31.3
Comorbidities (Known history)	No co-morbidity	111	62. 7
	Diabetes	22	12.5
	Hypertension	44	25
	HIV	12	6.8
	Others*	30	17
Duration of Hospital Stay	<3days	50	28.4
	3-5days	82	46.6
	>5days	39	22.2
Acute kidney Injury	No	61	34.5
	Yes	68	38.4
	Incomplete data	48	27.1
Hypokalemia	No	80	45.2
	Yes	81	45.8
	Mild (3.4-3.0mEq/L)	22	12.2
	Moderate (2.5-2.9mEq/L)	28	15.8
	Severe (<2.5mEq/L)	31	17.5
	Incomplete data	16	9.0
Hyponatremia	No	100	56.5
	Yes	61	34.5
	Mild (130-134mEq/L)	35	19.8
	Moderate (125-129mEq/L)	17	9.5
	Severe (<125mEq/L)	9	5.1
	Incomplete data	16	9.0
Hypochloremia	No	110	62.1
	Yes (<96mEq/L)	42	23.7
	Incomplete data	25	14.5
Outcomes	Resolved	163	93.7
	Died	11	6.25

*Old stroke, malignancy, bronchial asthma, psychiatric illness, seizure; AWD = Acute watery diarrhoea

Table 4: Factors associated with renal complications in patients with acute watery diarrhea admitted to Cholera Treatment Center of Yekatiti-12 Hospital Medical College, Addis Ababa, Ethiopia

Clinical characteristics		No AKI (%)	AKI (%)	AOR	95% CI	*p* value
Age group (years)	<15	12 (80.0)	3 (20.0)			1
	15-45	26 (45.6)	31 (54.4)	4.76	1.214-18.7	0.025*
	>45	23 (40.4)	34 (59.6)	5.9	1.501-23.3	0.01*
Comorbidity	No co-morbidity	44 (51.8)	17 (48.7)			1
	Comorbidity	17 (38.6)	27 (61.4)	1.7	0.812-3.57	0.15
Sex	Female	32 (53.5)	28 (46.7)			1
	Male	29 (42.0)	40 (58.0)	1.5	0.78-3.1	0.2
Length of stay	<3 days	38 (65.5)	20 (34.5)			1
	≥3 days	22 (33.3)	44 (66.7)	3.8	1.8-8.05	0.001*
Level of DHN	AWD with No DHN	11 (73.3)	4 (26.7)			1
	AWD with Some DHN	30 (52.6)	27 (47.4)	1.98	0.61-6.431	0.25
	AWD with Severe DHN	20 (35.1)	37 (64.8)	3.96	1.205-13.018	0.023*
Hyponatremia	No Hyponatremia	40 (54.4)	34 (45.5)			1
	Hyponatremia	20 (35.1)	37 (64.9)	1.85	1.45-27.83	0.014*
Hypochloremia	No Hypochloremia	47 (58.8)	33 (41.3)			1
	Hypochloremia	3 (9.7)	28 (90.3)	2.6	3.2-67.17	0.001*
Hypokalemia	No Hypokalemia	28 (49.1)	29 (50.9)			1
	Hypokalemia	25 (41.0)	36 (59.0)	1.34	0.925-15.9	0.06

AKI=Acute kidney injury; DHN = Dehydration; AWD= Acute watery diarrhea; AOR = Adjusted Odd Ratio; CI=Confidence Interval

Factors associated with renal complications in patients with suspected cholera:

The mortality rate among patients with AWD suspected of cholera and admitted to the CTC was 6.25% (Table 3). All patients who died in the CTC had acute kidney injury (AKI) and 90.1% of them had an admission diagnosis of AWD with severe DHN or shock. Over 38% of AWD patients admitted to CTC developed acute kidney injury (AKI). The risk of developing AKI was significantly increased with increased age, electrolyte disturbance, and level of dehydration.

The risk of AKI among older patients aged above 45 years of age was 5.9 times higher than younger patients aged less than 15 years (95% CI: 1.501 - 23.3, p =0.01). Patients staying longer than 3 days in the hospital had 3.8 times higher risk of AKI (95% CI: 1.8-8.05, p=0.001), and patients with severe dehydration had 3.96 times higher risk of AKI compared to patients with no dehydration (95% CI:1.205-13.018, p=0.023) (Table 4). Although the burden of AKI was higher among females and patients with comorbidities, the differences were not significantly different (p>0.05).

Frequency of risk factors for acute watery diarrhea in patients with suspected cholera:

Of all the AWD patients suspected of cholera and admitted to CTC of yekatit-12 Hospital Medical College, 76.7% of patients did not have the preceding overt contact history to diarrheal patient, 80.1% did not have attendance to social gathering, 92.6% did not have travel history in the preceding 10 days, and about 87% did not have similar history in the family. However, 54% had history of eating foods outside of home, 81.3% had history of eating raw fruits and vegetables, about 80% drunk water stored for over 3 days and not chlorinated or boiled (Table 5).

Discussion:

Frequent outbreaks of cholera continue to be one of the major public health problems in low-and-middle-income countries (LMICs) including Ethiopia where water, sanitation, and hygiene (WaSH) are inadequate (12). In this study, 176 patients with acute watery diarrhea suspected to have cholera were admitted to the cholera treatment center (CTC) at Yekatit-12 Hospital Medical College, of which females accounted for about 52%, and 90% were at least 15years of age. The age-specific attack rate was highest in the most productive age group of 15–44 years, which is consistent with different studies in Ethiopia (13,22) and beyond (23,24,26, 27,28,29,30).

The current outbreak occurred during the rainy season and involved all sub-cities of Addis Ababa. A similar pattern was also documented in previous episodes of the outbreaks in the capital (22). Due to the widespread outbreak of cholera across the regional states of Ethiopia (14,25) and the continuous flux of people to Addis Ababa from regional states, infections

Table 5: Frequency of common risk factors for acute watery diarrhoea among patients admitted to the Cholera Treatment Center of Yekatiti-12 Hospital Medical College, Addis Ababa, Ethiopia

Characteristics of risk factors		**Frequency**	**Percentage (%)**
Contact with diarrheal patient	No	135	76.7
	Yes	40	22.7
Prior attending of social gathering	No	141	80.1
	Yes	35	19.9
Prior travel history within 10days	No	163	92.6
	Yes	13	7.4
Similar history in the family	No	153	86.9
	Yes	23	13.5
History of eating food outside home	No	81	46.0
	Yes	95	54.0
Eating raw fruits and vegetables	No	12	6.8
	Yes	143	81.3
Where do you shop fruits and vegetables	Market	122	69.3
	Roadside	32	18.2
Common source of drinking water	Household tap water	107	60.0
	Municipality tap water	46	26.1
	Well water	21	11.9
	Bottled water	2	1.1
Storing drinking water in the container	No	28	15.9
	Yes	147	83.5
How frequent you get pipe water	<3days	21	11.9
	3-5days	94	53.4
	>5days	40	22.7
Chlorination or boiling of drinking water	No	150	85.2
	Yes	20	11.4
Type of toilet	No toilet	14	8.0
	Communal toilet	52	29.5
	Separate toilet	109	61.9
Type of latrine	Pit latrine	149	84.7
	Indoor toilet	12	6.8
Water and soap in the toilet for cleaning purpose	No	87	49.4
	Yes	75	42.6
Hand washing practice with soap after the toilet	No	10	5.7
	Yes, Some times	61	34.7
	Yes, always	94	53.4

might have been transmitted to the capital city. More than half of patients admitted to cholera treatment center had low level of education and low socio-economic status, consistent with the high burden of similar faeco-oral diseases among under privileged low socioeconomic population of the communities of Addis Ababa (9).

In the current study, the major symptoms among patients with AWD admitted to the CTC were watery diarrhea in 27.7%, and over 90% had both watery diarrhea and vomiting of ingested matter. This is consistent with previous data from Ethiopia (9,19,22), and cholera studies in other countries (21,26). Only 11.9% of the patients with AWD had an admission diagnosis of no dehydration while about half of the patients were diagnosed with some dehydration, and 36.7% had severe dehydration. Severity of dehydration observed in our study is similar to those reported in studies from Ethiopia (13,22), Iran (26), Pakistan (27), and Zambia (28). However, cases of severe dehydration were higher in our study than other studies from Ethiopia (19), and Iran (21), which may be due to the delayed presentation of patients, and only those patients with marked symptoms might have sought medical attention, which is consistent with the low health seeking behavior in the region.

Cholera is characterized by a devastat-

ing watery diarrhea which leads to rapid dehydration and death in 50 - 70% of untreated patients (27,29). Therefore, prompt and adequate rehydration is the corner stone of therapy for such acute water diarrheal patients (2,4). Oral rehydration salt (ORS) was given to patients with no dehydration, some dehydration, and also patients with severe dehydration while triaging and assessing the level of dehydration as well as during recovery phase. All patients with severe DHN were also given intravenous fluid of ringer's lactate, and antibiotics, which is consistent with similar reports (27,29). Unless for the treatment of sepsis including hospital acquired pneumonia on the discretion of the treating physician, 300mg stat dose of oral doxycycline was the antibiotic of choice to decrease the duration of illness and the period of infectiveness (4).

Many studies have reported that antibiotic therapy shortens the diarrheal duration by about a day, reduces the total stool volume by 50%, reduces the amount of rehydration fluids required by 40%, and reduces the mean duration of fecal excretion of bacteria by almost 3 days (2). Although intravenous fluids and antibiotics were reserved for patients with severe dehydration and/or some dehydration with profound fluid loss (2,4,29), significant number of patients with no dehydration were also given IV fluids, which contradicts the recommendations. The high mortality rate in cholera and renal as well as electrolyte complications might have influenced the choice of the treating physicians to adopt this aggressive approach.

In our current study, the proportion of acute kidney injury among AWD patients was 38.4%, and the risk of developing AKI was associated with increasing age (p=0.02), increased length of hospitalization (p=0.01), severity of dehydration (p=0.017), hyponatremia (p=0.013) and hypochloremia (p<0.01). The burden of AKI was similar to the finding in tertiary hospital of Pakistan (27) but much lower than the rate of 78% in Bangalore hospital in India, which might be due to limited number of cases, and severe disease in the latter setting where about 56.8% of patients had severe dehydration and over 90% had hypovolemic shock (30). We observed a significant electrolyte complication among the AWD patients admitted to CTC with hypokalemia, hyponatremia and hypochloremia in 45.8%, 34.5% and 23.7% of patients, respectively, which is in alignment with similar studies (21, 31).

The mortality rate among patients with AWD admitted to the CTC was 6.25%, which is comparable with other studies in Ethiopia (18), Sudan (32), and Syria (21). However, it is much higher than other retrospective studies in Ethiopia (13,19,22). The study design that reports actual real time burden as opposed to passively collected retrospective data (13,19,22), delayed presentation of patients to the referral center, severity of illness at presentation such as severe DNH or shock and AKI, and the presence of comorbidities might have contributed to the increased mortality in our study. The inaccessibility of hemodialysis for some of the critical patients with uremic complications might have also compounded the problem.

Cholera, a faeco-oral disease transmitted by consumption of contaminated food or drink, is one of the key indicators of poor socioeconomic development and remains a challenge to countries where access to safe drinking water and adequate sanitation cannot be guaranteed. Consistent with the notion that most of the cholera patients (80%) are asymptomatic but can transmit the infection (22), only 22.7% of our patients had overt contact with diarrheal patient and about 15% of patients had similar illness in the family. However, 54% of our patients' consumed fruits and vegetables which were commonly grown around the unhygienic rivers of the heavily populated Addis Ababa city where most of the household sewerage lines terminate (22). Astonishingly, about 90% of patients obtained their drinking water at most twice a week, and therefore utilize stored drinking water for days with only about 10% using chlorination or boiling before drinking. This finding is consistent with other similar studies in Ethiopia (9,17). Similarly, about 85% of our patients had shared pit latrine, 50% had no water and soap in the toilet for hand washing, and 40% do not practice hand washing or only sometimes wash their hands after using toilet. The extent of poor sanitation in the current setting is better than report from rural Ethiopia (18), but comparable with the study in Addis Ababa (9).

The main limitation of the study is its small sample size from a single center, which makes it difficult to generalize the result to the broader population. The cross-sectional design of the study can only establish associations and not cause-effect relationship between the predictors and the outcome variables. Due to financial constraints and lack of easy availability, some patients did not have laboratory panels including laboratory confirmation of cholera.

Conclusion:

The cholera outbreak in Addis Ababa, Ethiopia was characterized by moderate to severe dehydration, high burden of electrolyte imbalance, acute kidney injury and high case fata-

lity rate. Community WaSH intervention, early case detection, and coordinated management strategies play key roles in reduction of disease burden, morbidity and mortality.

Contributions of authors:

MM conceived the study, supervised the study, did the statistical analysis and interpretation, Written the manuscript, MR did statistical analysis, revised the manuscript. GC participated in data collection, did statistical analysis, and revised the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

Data availability:

Individual data will not be made publicly available for privacy protection. De-identified participant data will be shared upon reasonable request.

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Original Article **Open Access**

Nasal colonization of methicillin and glycopeptide resistant *Staphylococcus aureus* among outpatients at a clinic facility in Maputo, Mozambique: A cross-sectional study

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Abstract:

Background: Nasal colonization by *Staphylococcus aureus* is a major source of community and hospital associated staphylococcal infections, including methicillin-resistant *S. aureus* (MRSA) infections. Screening of MRSA nasal colonization is important in the prevention and control of infection and may provide useful information to guide antimicrobial therapy. The study aimed to determine the prevalence of nasal colonization by methicillin-resistant *S. aureus* and its glycopeptide susceptibility pattern among symptomatic and asymptomatic individuals in Maputo, Mozambique.
Methodology: A cross-sectional study of symptomatic outpatients and asymptomatic students at Eduardo Mondlane University in Maputo, was conducted by collecting nasal specimens for *S. aureus* isolation. Using culture techniques such as Mannitol salt and HiCrome™ Rapid MRSA agar, *S. aureus* was isolated based on the colonial characteristics and confirmed by Gram staining, catalase, and Microgen®Staph tests. The antibiotic susceptibility test was performed by the Kirby-Bauer disk diffusion method on Salt Mueller Hinton agar for methicillin (cefoxitin). Vancomycin and teicoplanin antibiotics were tested as an initial screening for glycopeptide inhibition patterns by the disc diffusion method.
Results: A total of 50 (39.1%) *S. aureus* isolates were obtained from nasal samples collected from 128 participants, of whom 104 (31.3%) were from university students and 24 (7.8%) from outpatients in the university clinic. The prevalence of MRSA (resistance to cefoxitin) in the study population was 7.8% (n=24/128). For the glycopeptides, 14.1% and 11.7% showed reduced susceptibility to teicoplanin and vancomycin, respectively.
Conclusion: In this study, the prevalence of nasal colonization by S. aureus was 39%, while the prevalence of MRSA was relatively low (7.8%) in the poplution studied. However, the reduced glycopeptide susceptibility highlights the need for surveillance, to prevent the spread of potential glycopeptide-resistant *S. aureus* in vulnerable populations.

Keywords: *Staphylococcus aureus,* Nasal colonization, antibiotic susceptibility, Maputo.

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Colonisation nasale par *Staphylococcus aureus* résistant à la méthicilline et profil de sensibilité aux glycopeptides chez des individus symptomatiques et asymptomatiques à Maputo, Mozambique

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Résumé:

Contexte: La colonisation nasale par *Staphylococcus aureus* est une cause majeure d'infections staphylococciques

communautaires et nosocomiales, notamment d'infections à *S. aureus* résistant à la méthicilline (SARM). Le dépistage de la colonisation nasale par le SARM est important pour la prévention et le contrôle de l'infection et peut fournir des informations utiles pour orienter l'antibiothérapie. L'étude visait à déterminer la prévalence de la colonisation nasale par le SARM et son profil de sensibilité aux glycopeptides chez des individus symptomatiques et asymptomatiques à Maputo, au Mozambique.

Méthodologie: Une étude transversale a été menée auprès de patients externes symptomatiques et d'étudiants asymptomatiques de l'Université Eduardo Mondlane de Maputo, par prélèvement nasal en vue de l'isolement de S. aureus. L'isolement de *S. aureus* a été réalisé par culture sur gélose au mannitol et au sel et sur milieu HiCrome™ Rapid MRSA, en se basant sur les caractéristiques des colonies. L'identification a été confirmée par coloration de Gram, test de la catalase et test Microgen® Staph. La sensibilité aux antibiotiques a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer sur gélose Mueller-Hinton salée pour la méthicilline (céfoxitine). La vancomycine et la teicoplanine ont été testées par la méthode de diffusion sur disque pour évaluer l'inhibition de la croissance par les glycopeptides.

Résultats: Cinquante isolats de *S. aureus* (39,1%) ont été obtenus à partir d'échantillons nasaux prélevés chez 128 participants, dont 104 (31,3%) étaient des étudiants et 24 (7,8%) des patients externes d'un centre hospitalier universitaire. La prévalence du SARM (résistance à la céfoxitine) dans la population étudiée était de 7,8% (n=24/128). Concernant les glycopeptides, 14,1% et 11,7% des souches ont présenté une sensibilité réduite à la teicoplanine et à la vancomycine, respectivement.

Conclusion: Dans cette étude, la prévalence de la colonisation nasale par *S. aureus* était de 39 %, tandis que la prévalence du SARM était relativement faible (7,8 %) au sein de la population étudiée. Toutefois, la sensibilité réduite aux glycopeptides souligne la nécessité d'une surveillance accrue afin de prévenir la propagation de *S. aureus* potentiellement résistant aux glycopeptides aux populations vulnérables.

Mots-clés: *Staphylococcus aureus*, colonisation nasale, sensibilité aux antibiotiques, Maputo

Introduction:

Staphylococcus aureus (S. aureus) is a versatile commensal microorganism found in the human body and is associated with a wide variety of infections with high morbidity, mortality, and healthcare-associated costs (1,2). *S. aureus* can efficiently adapt to multiple niches, establishing solid interactions with epithelial cells (2,3) and able to overcome these defenses and colonize this microenvironment (2,4,5). The most frequent carriage site is the nose, with approximately 20 to 40% of the general population being permanently but asymptomatically colonized, and another 30% carrying the bacterium intermittently (2,6).

Intermittent nasal colonization by *S. aureus* represents the major risk factor for infections such as cellulitis, endocarditis, impetigo, pneumonia, urinary tract infections, osteomyelitis, pneumonia, septicemia, and toxic shock syndrome (TSS) (2,4,7,8). Its pathogenicity is multifaceted, encompassing antimicrobial resistance (AMR) and a high proficiency for biofilm formation (2,9,10). *S. aureus* has become resistant to various antimicrobial agents, including the commonly used penicillin-related antibiotics such as methicillin, designated methicillin-resistant *S. aureus* (MRSA). It is considered one of the principal pathogens responsible for 100,000 global deaths annually associated with AMR (11). For instance, most MRSA isolates have also shown resistance to vancomycin, a glycopeptide that was introduced and widely used as a preferred option for treating MRSA infections (12–14).

Globally, including in the African context, the prevalence rates of MRSA and multidrug-resistant (MDR) *S. aureus* strains reach as high as 61.6% (15–22). However, data from sub-Saharan Africa, particularly in Mozambique, remain scarce. A few studies conducted in both hospital and community settings have reported *S. aureus* infection rates of up to 12.3% (22, 23), with MDR and MRSA accounting for as much as 33.3% of these infections (23–25). This has escalated into a global health challenge, as bacterial infections once easily treated have become increasingly difficult to manage (17,26).

Nasal colonization of both *S. aureus* and MRSA plays a key role in the epidemiology and pathogenesis of both MDR *S. aureus* and MRSA infections in various populations (13,17,27). Screening for and eliminating nasal carriage of *Staphylococcus aureus* and MRSA has been associated with a reduction in infection rates (28–30). On the other hand, understanding antimicrobial patterns of *S. aureus* is crucial for developing effective prevention and control measures, particularly in developing countries, where healthcare infrastructure and access to medical services remain limited (13,30,31).

This study therefore aimed to estimate the prevalence of nasal colonization by *S. aureus* and its methicillin-resistance profile among symptomatic and asymptomatic individuals in Maputo, Mozambique, while additionally characterizing the glycopeptide susceptibility patterns of the isolates.

Materials and method:

Study type and population:

This was a cross-sectional study carried out from June to November 2022 (during Covid-19 pandemic), in a group of symptomatic outpatients attending the Eduardo Mondlane University (EMU/UEM) clinic, and asymptomatic students from the Department of Biological Sciences, Eduardo Mondlane University (DBC-EMU/UEM).

Study participants and sample collection:

Nasal specimens were collected aseptically from 128 participants, comprising outpatients (n=24) and students (n=104). Willingness of the participants to participate in the study was a strong criterion for selection. Additionally, participants were asked if they had undergone antibiotic treatment within the last two weeks prior to sample collection; and those who had received were excluded. The nasal specimens were collected with labelled sterile swab sticks and taken within 2 hours of collection for inoculation on selective media in the Microbiology laboratory at the DBC-EMU/UEM.

Ethical approval:

Before the study, ethical approval was obtained from the Mozambican National Ethical Committee on June 06, 2022 (Approval No. 347/CNBS/22). The study was explained to the participants, and only those who provided written informed consent and informed assent in the case of minors under 18 years of age, were included. Confidentiality and privacy were maintained throughout the study.

Isolation and characterization of *S. aureus:*

Each of the swab samples was inoculated onto sterilized Mannitol salt agar (MSA) and HiCrome™ Rapid MRSA agar (HiMedia, India) plates and incubated aerobically at 37°C for 24 to 48 hours. The culture media were prepared and sterilized according to the manufacturer's instructions. The isolates were characterized using standard established microbiological methods.

Isolates with golden yellow-coloured colonies on MSA agar plates and greenish yellow-coloured colonies on HiCrome™ Rapid MRSA agar were presumptively identified as *S. aureus*, and subsequently sub-cultured on MSA plates to obtain pure colonies, followed by confirmation by Gram staining, catalase, and agglutination (Microgen®Staph) tests. All isolates that were Gram positive cocci (grape-like clusters) and positive for catalase and agglutination tests, were confirmed as *S. aureus* and submitted for antibiotic susceptibility testing (AST).

Antibiotic susceptibility testing:

Antibiotic susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (32), and from previous studies by Roy et al., (33) and Deepak et al.,(34). The testing was performed using the Kirby-Bauer disk diffusion method on Mueller Hinton agar (MHA) supplemented with salt (HiMedia, India) for detection of methicillin resistance in *S. aureus* isolates using cefoxitin. Vancomycin and teicoplanin discs were additionally included as a preliminary screening approach to observe glycopeptide inhibition patterns among the isolates, while recognizing that definitive susceptibility categorization for glycopeptides is recommended using minimal inhibitory concentration (MIC)-based methods (broth microdilution or E-test) (35,36).

Briefly, a suspension of colonies of the pure culture of *S. aureus* isolate was prepared with normal sterile saline solution and adjusted to a turbidity of 0.5 McFarland standards. The cell suspension was inoculated with a sterile swab onto the surface of MHA and left briefly to dry at room temperature. Three antibiotic discs (Oxoid, UK): cefoxitin (30µg), vancomycin (30 µg) and teicoplanin (30µg) were placed on the surface of the inoculated MHA plates, and allowed to stand for at least 30 minutes before incubation for 24 hours at 30-35°C, for the growth of methicillin resistant strains (26,37).

The diameter of the inhibition zone (in mm) of the isolate was measured (in mm) with a ruler. The interpretation of AST result as sensitive, intermediate or resistant was in accordance with CLSI guidelines, as the shown in Table 1. MRSA strain (ATCC 25923), provided by José Sumbana (Laboratory of Microbiology, Faculty of Medicine, UEM), was used as a control.

Table 1: Antibiotic discs and the inhibition zone diameters used for the measurement/interpretation of the results

Antibiotic disc contents	Zone diameter of inhibition (mm)			References
	R (resistant)	I (intermediate)	S (sensitive)	
Cefoxitin (30µg)	≤ 21	-	≥ 22	CLSI (33,35)
Vancomycin (30µg)	-	-	≥15*	Roy et al., (34)
Teicoplanin (30µg)	-	-	≥14*	CLSI (33), Deepak et al., (37)

*All *S. aureus* isolates with inhibition zones below the specified threshold, which is indicative of reduced antimicrobial susceptibility, were re-tested to confirm the observed results; CLSI – Clinical and Laboratory Standard Institute.

Statistical analysis:

Statistical data analysis was performed using SPSS 21 software (IBM Corp., Armonk, NY). Frequencies were obtained and percentages were calculated for study variables. Demographic characteristics were compared with the use of the Chi-square test. All reported *p*-values of less than or equal to 0.05 were considered to be statistically significant ($p<0.05$).

Results:

Nasal samples were collected from 128 participants: 24 outpatients from EMU/UEM clinic representing the symptomatic participants, and 104 were students from DBC-EMU/UEM, representing the asymptomatic participants. The modal age group for both groups was 18–30 years with a total of 116 participants. The age range of the participants was 1–62 years (Table 2).

Distribution of *S. aureus* and CoNS by participant's characteristics:

A total of 50 (39.1%) out of 128 participants had *S. aureus* isolated from their nasal samples while 54 (42.2%) had coagulase-negative *Staphylococcus* (CoNS) isolated. EMU/UEM clinic outpatients had higher colonization rates by *S. aureus* (41.7%, n=10/24) and CoNS (45. 8%, n=11/24) than the DBC-EMU/UEM students with *S. aureus* rate of 38.5% (n=40/104) and CoNS rate of 41.3% (n=43/104) respetively, but the differences were not statistically significant ($p=0.81$).

Regarding the sex distribution, male participants had higher *S. aureus* colonization rate (57.5%, n=27/47) than female participants (28.4%, n=23/81) ($p=0.002$), while the female participants had higher CoNS colonization rate (50.6%, n=41/81) than the male participants (27.7%, n=13/47) ($p=0.02$). No statistically significant difference was found for *S. aureus* ($p=0.153$) and CoNS ($p=0.285$) colonization prevalence between the age groups.

Table 2: Prevalence of *S. aureus* and coagulase-negative *Staphylococcus* nasal colonization among the study population

Category	Sample Number (N)	*S. aureus*		*P* value	CoNS		*P* value
		N	(%)		n	(%)	
Overall	128	50	39.1	-	54	42.2	-
Type of participants							
EMU/UEM clinic Outpatients	24	10	41.7	0.819	11	45.8	0.819
DBC-EMU/UEM Students	104	40	38.5		43	41.3	
Sex							
Female	81	23	28.4	0.002*	41	50.6	0.02*
Male	47	27	57.5		13	27.7	
Age group (years)							
≤17	2	2	100.0	0.153	0	0.0	0.285
18-30	116	42	36.2		51	43.9	
31-45	4	3	75.0		1	25.0	
46-60	3	2	66.7		0	0.0	
≥61	3	1	33.3		2	66.7	

DBC – Department of Biological Sciences; EMU/UEM – Eduardo Mondlane University; CoNS – Coagulase-Negative *Staphylococcus*; *statistically significant

Distribution of MRSA and isolates with reduced zone of inhibition for glycopeptides:

Table 3 presents the frequencies of MRSA and *S. aureus* isolates with observed reduced zones of inhibition for glycopeptides. Methicillin resistance, assessed using cefoxitin, was detected in 7.8% of the study population (n=10/128) and 20.0% of *S. aureus* isolates (n=10/50), occurring in 10.0% (n=1/10) of *S. aureus* from outpatients and 22.5% (n=9/40) of *S. aureus* from students (p=0.6631). Reduced vancomycin susceptibility was more frequent, with 30.0% among *S. aureus* isolates (n=15/50) and 11.7% among the study population (n=15/128), showing inhibition zones below the adopted threshold for vancomycin, including 40.0% (n=4/10) among *S. aureus* isolates from outpatients and 27.5% (n=11/40) among *S. aureus* isolates from students (p=0.4616).

Similarly, reduced teicoplanin susceptibility was also frequent, with 36.0% among *S. aureus* isolates (n=18/50) and 14.1% among the study population (n=18/128), exhibiting inhibition zones below the adopted threshold for teicoplanin, with higher (but insignificant) rates in outpatients (60.0%, n=6/10) compared with students (30.0%, n=12/40) (p=0.1381).

The simultaneous detection of methicillin resistance and reduced glycopeptide susceptibility in *S. aureus* isolates was also evaluated (Fig 1). Overall, 28.0% of the isolates (n=14/50) demonstrated decreased susceptibility to at least two of the antibiotics tested. Among these, 12.0% (n=6/14) showed reduced susceptibility to methicillin with either one of the glycopeptides, 4.0% (n=2/14) showing reduced susceptibility to all three antibiotic agents (methicillin, vancomycin, and teicoplanin), and 12.0% (n=6/14) exhibiting reduced susceptibility specifically to both glycopeptides (vancomycin and teicoplanin).

Table 3: Frequencies of MRSA and *S. aureus* isolates with reduced zone of inhibition to glycopeptides

Antibiotic disc contents and susceptibility profile/pattern	Overall (n=50)		EMU/UEM clinic Outpatients (n=10)		DBC-EMU/UEM Students (n=40)		*p* value
	Number	(%)	Number	(%)	Number	(%)	
Methicillin-resistance							
Cefoxitin (30µg)	10	20.0	1	10.0	9	22.5	0.6631
Reduced zone of inhibition/susceptibility for glycopeptides							
Vancomycin (30µg)	15	30.0	4	40.0	11	27.5	0.4616
Teicoplanin (30µg)	18	36.0	6	60.0	12	30.0	0.1381

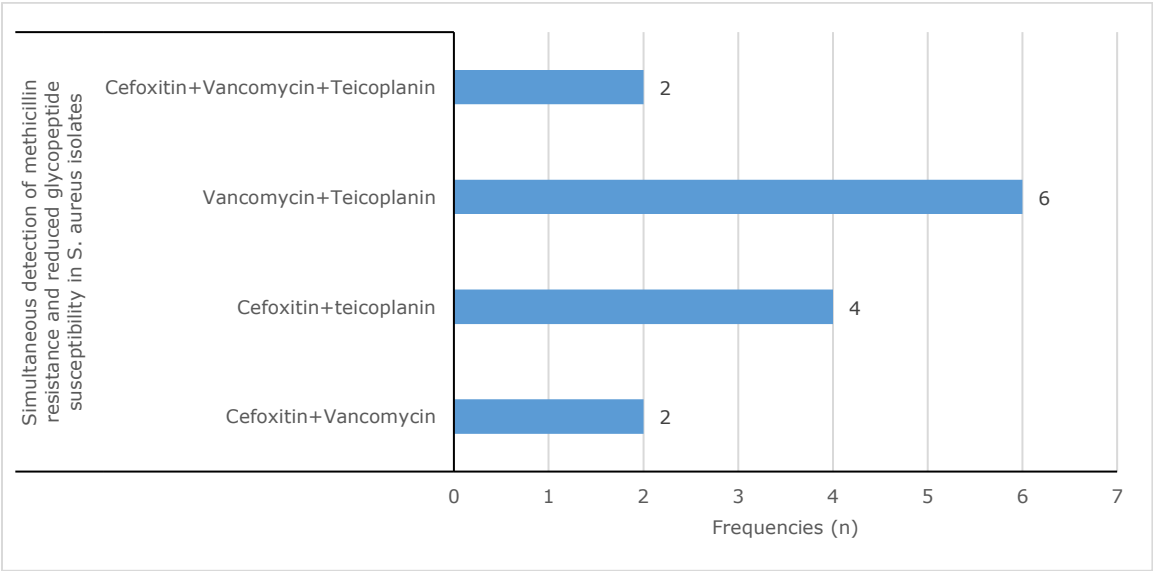

Fig 1: Frequency of simultaneous methicillin resistance and reduced glycopeptide susceptibility in *S. aureus* isolates

Discussion:

Staphylococcus aureus colonization has long been recognized as an important risk factor for subsequent infection caused by the colonizing strain (16,27). This pathogen can cause a wide range of clinical manifestations, from minor skin and soft tissue infections to severe invasive diseases such as pneumonia, bacteraemia, and sepsis. Its clinical relevance is further heightened by its remarkable capacity to acquire resistance to multiple classes of antibiotics (26,33). Of particular concern is the global spread of multidrug-resistant (MDR) strains, especially MRSA, which complicates treatment and reduces available therapeutic options. Nasal colonization of *S. aureus* has been implicated in both community-associated infections, such as skin and soft tissue infections, and hospital-associated infections, including bacteremia and surgical site infections (17,27,38).

The present study assessed the prevalence of nasal carriage of *S. aureus* and MRSA among symptomatic and asymptomatic individuals, while also examining glycopeptide inhibition zone patterns that may suggest reduced susceptibility. Our findings demonstrate an overall *S. aureus* nasal carriage rate of 39.1%, which aligns with previous studies conducted in other geographic regions. For instance, Onanuga and Temedie (26) in Nigeria, Kuehnert et al. (39) in the United States and Uemura et al. (40) in Japan, reported prevalence rates of 33.3%, 32.4% and 36.0%, respectively, among healthy adults. These findings support the notion that approximately one-third of the population may carry *S. aureus* in the anterior nares across different populations.

Nevertheless, variations in prevalence have been reported. Higher colonization rates exceeding 43% were documented in both hospital and non-hospital populations in Abia State, Nigeria (41), as well as among medical students (42) and hospital personnel (43) in Nepal. In contrast, substantially lower colonization rates have also been observed. For instance, Adesida et al., (44) reported a prevalence of only 14.0% among students in Lagos, Nigeria, while Abie et al., (45)found 23.2% prevalence among janitors in Addis Ababa, Ethiopia. The recent study by Taqveem et al., (46) in Pakistan similarly noted low prevalence among students. Such discrepancies highlight that nasal carriage is influenced by multiple epidemiological and environmental factors, including antibiotic usage, healthcare exposure, population demographics, and even sampling and laboratory techniques (17, 26).

Antibiotic exposure appears to be particularly influential. Populations receiving antibiotic treatment at the time of sampling often exhibit lower *S. aureus* prevalence due to suppression of colonization by susceptible strains. Conversely, hospital settings typically report higher colonization rates because of increased opportunities for bacterial transmission in environments with high infection burdens (44). In contrast, non-hospitalized populations not under antibiotic treatment may exhibit relatively higher prevalence, reflecting natural colonisation patterns. This has been confirmed in studies of community populations where nasal colonization typically ranges between 20–30% (2,6). These findings collectively demonstrate that *S. aureus* colonization is shaped by a complex interplay between microbial ecology, host factors, and healthcare exposure.

In the present study, the anterior nares were confirmed as a major reservoir for *S. aureus*, consistent with previous reports (27). Importantly, we found no significant difference in colonization rates between symptomatic (outpatients) and asymptomatic individuals (students) (p=0.819), suggesting that carriage is not necessarily associated with acute infection risk at the individual level. However, colonisation serves as a latent risk, providing a reservoir for potential infection and onward transmission. Interestingly, colonization was significantly associated with sex (p=0.002), with males exhibiting higher carriage rates (57.5%) compared to females (28.4%). This observation echoes findings by Taqveem et al., (46), where male students demonstrated higher prevalence of both *S. aureus* (57.7%) and MRSA (21.4%) compared to females (42.3% and 17.9%). Potential explanations include behavioral differences (e. g., hygiene practices, sports participation, or crowding exposures), as well as biological differences such as hormonal or immune factors.

Age-related colonization patterns have also been described globally, with higher rates observed shortly after birth and during adolescence (16). In African populations, additional risk factors have been identified, including HIV infection, frequent handwashing with harsh detergents, rural residence, and hospitalization, particularly in surgical wards (47,48). Conversely, higher educational status and male sex have been reported as protective in some settings (16), suggesting that risk factors may be context-specific and interact with socioeconomic, environmental, and cultural variables.

Antibiotic susceptibility testing in our study revealed low overall methicillin-resistance rates; 20.0% of the nasal *S. aureus* isolates, representing 7.8% of the study population. These findings are consistent with both local and

inter- national studies. For example, Ceccarelli et al., (49) reported 4.0% MRSA prevalence, van der Meeren et al., (23) found 15.1%, and Vubil et al., (24) documented 8.0% in Mozambique. Comparable results were also seen in Pakistan, with Taqveem et al., (46) reporting 5.5%. While MRSA prevalence appears relatively low in healthy carriers, its presence remains clinically significant because colonization can lead to difficult-to-treat infections, especially in healthcare environments.

Comparisons with international studies underscore regional variability. Chen and Huang (50) reported MRSA prevalences from <5% to >35% in Asian countries, Olonitola et al., (51) 14.85% in Nigeria, Rijal et al., (52) 56.1% in Nepal, and Onanuga and Temedie (26) 27.5% in Nigeria. The variability may reflect differences in infection control practices, antibiotic prescribing patterns, and genetic background of circulating strains. Notably, our MRSA estimation was based on phenotypic resistance to cefoxitin rather than molecular detection of the *mecA* gene, which encodes the altered penicillin-binding protein (PBP2a). This methodological limitation means that some "phenotypic MRSA" isolates may not carry *mecA*, as reported in previous studies (49,53).

The public health implications of these findings are profound. Nasal carriage of *S. aureus*, particularly MRSA, is a major driver of subsequent infections, with estimates suggesting 7% of hospitalized patients and 2% of community populations carry MRSA (17). Furthermore, healthcare-associated MRSA strains often display extensive multidrug resistance, whereas community-acquired strains remain more susceptible to non-β-lactam antibiotics (54). This distinction is critical for guiding empirical therapy and infection control interventions.

Beyond methicillin resistance, reduced inhibition zones to glycopeptides were also observed among several isolates. Overall, 30.0% (11.7% of the study population) and 36% (14.1% of the study population) of *S. aureus* isolates showed inhibition zones below the adopted threshold for vancomycin and teicoplanin, respectively. These findings warrant attention because glycopeptides remain key therapeutic agents for MRSA infections (26,55). However, the use of disc diffusion for glycopeptide susceptibility testing has limitations as these large molecules diffuse poorly in agar and may lead to underestimation or misclassification of resistance (56).

While most studies report low glycopeptide resistance (≤11.1%), usually reflecting intermediate susceptibility phenotypes (34,55), higher rates of intermediate vancomycin resistance have been reported in African settings (≤14.5%) (14,57). The emergence of vancomycin-resistant *S. aureus* (VRSA) remains a serious global concern, particularly because vancomycin has long served as a last-line therapy against MRSA infections, with teicoplanin increasingly used as an alternative (26,34,55). These observations highlight the importance of continued surveillance and improved laboratory capacity for confirmatory testing in resource-limited settings such as Mozambique.

This study has some limitations. First, susceptibility to vancomycin and teicoplanin was tested using disc diffusion, which is not recommended by the current CLSI guidelines (35–37,58). Therefore, the classification of isolates as presumptively resistant should be interpreted cautiously and ideally confirmed using MIC-based methods such as broth microdilution or E-test (35–37). Nevertheless, previous studies suggest that disc diffusion and E-test methods may yield broadly comparable results, supporting the pragmatic use of disc diffusion in laboratories with limited resources (34). Secondly, the relatively small and specific study population limits the generalizability of the findings, emphasizing the need for larger studies involving more diverse populations across Mozambique.

Conclusion:

The findings of this study underscore the importance of nasal carriage of *S. aureus* and MRSA as key reservoirs for subsequent infection and transmission. With carriage rates of 39.1% for *S. aureus* and 7.8% for MRSA in the study population, alongside reduced glycopeptide susceptibility, our findings highlight both the persistent burden of colonization and the growing challenge of antimicrobial resistance in Mozambique. Addressing these threats requires strengthened surveillance, efficient antibiotic stewardship, and reliable confirmatory testing. Future investigations should expand to larger cohorts, integrate molecular approaches for resistance detection, and assess the effectiveness of decolonization strategies in lowering infection risk.

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Contributions of authors:

All authors made significant contributions to the conceptualization, design and implementation of the study, and to the drafting of the manuscript.

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Conflicts of interest:

No conflict of interest is declared

Data availability:

The data that support the findings made in this study can be made available from the corresponding author on request.

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Original Article **Open Access**

Prevalence of *mecA* and *vanA* genes in nasal *Staphylococcus aureus* isolates from hospitalized patients in Babcock University Teaching Hospital, Ilishan-Remo, Nigeria

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Abstract:

Background: Antimicrobial resistance (AMR) among *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA), poses a major global public health challenge. Data on the molecular epidemiology of resistance genes in Nigeria remain limited. The objectives of this study are to determine the prevalence of *mecA*, *vanA*, and *vanB* genes among nasal *S. aureus* isolates, assess antimicrobial susceptibility profiles, and explore associations of prevalence with demographic and clinical characteristics of hospitalized patients in Babcock University Teaching Hospital (BUTH), Ilishan-Remo, Nigeria.
Methodology: This descriptive cross-sectional study was conducted among 400 inpatients admitted to BUTH, Ilishan-Remo, Nigeria for various medical conditions, between June 2023 and June 2024. Sociodemographic and clinical data were obtained through interviewer-administered questionnaires and structured data extraction tools. Nasal swabs were collected from consenting participants who has been hospitalized for at least 48–72 hours. *S. aureus* isolates were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method following CLSI 2023 guidelines. Polymerase chain reaction (PCR) was used to detect *mecA*, *vanA*, and *vanB* genes. Data were analysed using SPSS version 25.0. Associations between categorical variables were tested using Chi-square test and p value < 0.05 was considered statistically significant.
Results: *S. aureus* isolates were recovered from 27 (6.8%) of the 400 participants. *mecA* gene was detected in 25 of the 27 isolates (92.6%), representing 6.3% MRSA prevalence among the study participants, while *vanA* was identified in 7 of the 27 isolates (25.9%), representing 1.8% VRSA prevalence among the participants. No isolate carried *vanB* gene. Ward type showed a significant association with *mecA* carriage (χ^2=15.336, p=0.032), while demographic characteristics and prior antibiotic use were not significantly associated with resistance gene carriage. All isolates were susceptible to linezolid, tedizolid, ceftaroline, and quinupristin–dalfopristin. High resistance rates were observed for levofloxacin (63.0%), erythromycin (44.4%), and gentamicin (37.0%).
Conclusion: This study reports a high prevalence of methicillin (*mecA*) and vancomycin (*vanA*) resistance genes carriage among *S. aureus* isolates colonizing the nostrils of hospitalized patients in BUTH, Ilishan-Remo, Ogun State, Nigeria. Strengthened infection prevention measures, antimicrobial stewardship, and confirmatory molecular sequencing are urgently needed.

Keywords: *Staphylococcus aureus*, *mecA*, *vanA*, nasal colonization, antimicrobial resistance, Nigeria

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Prévalence des gènes *mecA* et *vanA* chez les isolats nasaux de *Staphylococcus aureus* provenant de patients hospitalisés au Centre hospitalier universitaire Babcock, Ilishan-Remo, Nigéria

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Résumé:

Contexte: La résistance aux antimicrobiens (RAM) chez *Staphylococcus aureus*, en particulier chez les souches résistantes à la méthicilline (SARM) et résistantes à la vancomycine (VRSA), constitue un enjeu majeur de santé publique à l'échelle mondiale. Les données sur l'épidémiologie moléculaire des gènes de résistance au Nigéria restent limitées. Cette étude vise à déterminer la prévalence des gènes *mecA*, *vanA* et *vanB* parmi les isolats nasaux de *S. aureus,* à évaluer les profils de sensibilité aux antimicrobiens et à explorer les associations entre cette prévalence et les caractéristiques démographiques et cliniques des patients hospitalisés au Centre hospitalier universitaire Babcock (BUTH), à Ilishan-Remo, au Nigéria.
Méthodologie: Cette étude descriptive transversale a été menée auprès de 400 patients hospitalisés au BUTH d'Ilishan-Remo, au Nigéria, pour diverses pathologies, entre juin 2023 et juin 2024. Les données sociodémographiques et cliniques ont été recueillies au moyen de questionnaires administrés par un enquêteur et d'outils structurés d'extraction de données. Des prélèvements nasaux ont été effectués chez les participants consentants, hospitalisés depuis au moins 48 à 72 heures. Les isolats de Staphylococcus aureus ont été identifiés par les méthodes microbiologiques standard. L'antibiogramme a été réalisé par la méthode de diffusion sur disque de Kirby-Bauer, conformément aux recommandations CLSI 2023. La réaction en chaîne par polymérase (PCR) a été utilisée pour détecter les gènes *mecA*, *vanA* et *vanB*. Les données ont été analysées à l'aide du logiciel SPSS version 25.0. Les associations entre les variables catégorielles ont été testées par le test du χ^2 et une valeur $p < 0,05$ a été considérée comme statistiquement significative.
Résultats: Des isolats de *S. aureus* ont été retrouvés chez 27 (6,8%) des 400 participants. Le gène *mecA* a été détecté dans 25 des 27 isolats (92,6%), soit une prévalence de SARM de 6,3% parmi les participants à l'étude, tandis que le gène *vanA* a été identifié dans 7 des 27 isolats (25,9%), soit une prévalence de VRSA de 1,8% parmi les participants. Aucun isolat ne portait le gène vanB. Le type de service hospitalier était significativement associé au portage du gène *mecA* (χ^2=15,336, p=0,032), tandis que les caractéristiques démographiques et les antécédents d'utilisation d'antibiotiques n'étaient pas significativement associés au portage de gènes de résistance. Tous les isolats étaient sensibles au linézolide, au tédizolide, à la ceftaroline et à la quinupristine-dalfopristine. Des taux de résistance élevés ont été observés pour la lévofloxacine (63,0%), l'érythromycine (44,4%) et la gentamicine (37,0%).
Conclusion: Cette étude révèle une forte prévalence du portage des gènes de résistance à la méthicilline (*mecA*) et à la vancomycine (*vanA*) chez les souches de *S. aureus* colonisant les narines de patients hospitalisés au BUTH d'Ilishan-Remo, dans l'État d'Ogun, au Nigéria. Le renforcement des mesures de prévention des infections, la gestion des antimicrobiens et le séquençage moléculaire de confirmation sont nécessaires de toute urgence.

Mots-clés: *Staphylococcus aureus*, *mecA*, *vanA*, colonisation nasale, résistance aux antimicrobiens, Nigéria

Introduction:

The global rise of antimicrobial resistance (AMR) represents one of the most pressing public health threats of the 21st century. Low-and-middle-income countries (LMICs) bear a disproportionate burden due to limited diagnostic capacity, weak antimicrobial stewardship, and high rates of infectious diseases (1).

Staphylococcus aureus remains a leading cause of hospital- and community-acquired infections worldwide, causing conditions ranging from mild skin and soft tissue infections to life-threatening diseases such as pneumonia, endocarditis, and sepsis (2). The emergence of methicillin-resistant *S. aureus* (MRSA), primarily mediated by *mecA* gene located on the staphylococcal cassette chromosome mec (SCC*mec*) has severely complicated treatment. MRSA is resistant to nearly all β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems (3). The global prevalence of MRSA varies considerably, with rates exceeding 50% in many hospital settings in Asia, Latin America, and Africa (4).

More recently, vancomycin-resistant *S. aureus* (VRSA) has emerged as a critical threat. Vancomycin has traditionally been the last-resort antibiotic for serious MRSA infections. VRSA strains often carry the *vanA* gene, which was originally described in *Enterococcus* species and confers high-level resistance to vancomycin and teicoplanin through alteration of the peptidoglycan precursor terminal D-alanyl-D-alanine to D-alanyl-D-lactate (5,6). The acquisition of both *mecA* and *vanA* genes by *S. aureus* produces a highly-resistant pathogen with extremely limited therapeutic options, significantly increasing morbidity and mortality of infections caused by these strains (7). Although VRSA remains rare globally, with fewer than 100 confirmed cases reported worldwide, its emergence in hospital environments with poor infection control practices is a sentinel event (8).

In Nigeria, the burden of AMR is substantial but poorly characterised (9). Several

studies have reported MRSA prevalence rates ranging from 30% to 80% among clinical isolates (10,11). However, most surveillance efforts have relied solely on phenotypic testing, and few have systematically examined the molecular epidemiology of resistance genes (12,13). There is a particular scarcity of data on *vanA* carriage among *S. aureus* in Nigeria, with most published studies reporting rates below 5% (14, 15). Understanding the molecular patterns of resistance and their association with patient demographics and clinical settings is essential for designing targeted interventions, strengthening infection prevention and control (IPC) policies, and informing empirical therapy guidelines.

Nasal colonisation with *S. aureus* is a well-established risk factor for subsequent infection, particularly in surgical patients and immunocompromised hosts (16), who also have the capacity to transmit *S. aureus* in the healthcare setting. This study aimed to determine the prevalence of *mecA*, *vanA* and *vanB* genes in *S. aureus* isolates colonizing the nostrils of inpatients in Babcock University Teaching Hospital (BUTH), a tertiary hospital in Nigeria, to assess antimicrobial susceptibility profiles, and to explore associations with demographic and clinical variables, including ward type and recent antibiotic use.

Materials and method:

Study design and setting:

This was a descriptive cross-sectional study integrating laboratory investigations and retrospective review of patient case notes. The study was conducted at BUTH, a 250-bed tertiary healthcare facility located in Ilishan-Remo, Ogun State, Nigeria, between June 2023 and June 2024. BUTH provides multidisciplinary services, including medical, surgical, paediatric, obstetric, and critical care.

Eight hospital wards were purposively selected to ensure representation across diverse clinical settings and antimicrobial prescribing patterns; Male Medical Ward, Female Medical Ward, Male Surgical Ward, Female Surgical Ward, Obstetric Ward, Paediatric Ward, Accident and Emergency (A &E) Unit, and Intensive Care Unit (ICU).

Ethical considerations:

Ethical approval was obtained from the Babcock University Health Research Ethics Committee (BUHREC 613/23). Written informed consent was obtained from all participants for prospective sample collection. A waiver of informed consent was granted for the retrospective component of the study.

Study population and sampling:

The study population included hospitalized individuals who had spent a minimum of 48 to 72 hours in the facility. Exclusion criteria were: patients with nasogastric tubes; individuals with incomplete records or those discharged before 48 hours of admission. A census sampling approach was used for retrospective data extraction, while 400 consenting inpatients were recruited for nasal swab collection. Overall, 421 eligible patients were identified, of whom 21 were excluded, leaving 400 participants enrolled.

Data collection:

Sociodemographic and clinical data (age, sex, marital status, educational level, occupation, ward of admission, prior antibiotic use within the past four months, and chronic medical conditions) were obtained through interviewer-administered questionnaires and structured data extraction tools. For the retrospective arm, data were sourced from case notes, medication charts, nursing records, and laboratory reports.

Specimen collection and laboratory analysis:

Nasal swab specimens were collected from participants on the third day of admission using sterile swabs transported in peptone water. Swabs sent to the medical microbiology laboratory of BUTH were processed within two hours and inoculated onto Mannitol Salt Agar for selective isolation of *S. aureus*. Isolates were identified based on colony morphology, Gram staining, catalase test, coagulase test, and confirmed using polymerase chain reaction assay.

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing of *S. aureus* isolates was performed using the modified Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines (17). The antibiotics tested included cefoxitin (30µg), teicoplanin (30 µg), ceftaroline (30µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), levofloxacin (5µg), clindamycin (2µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), quinupristin-dalfopristin (15µg), linezolid (30µg), tedizolid (2µg), and telavancin using E-test strips (0.002–32µg/mL). Inoculum density was standardized to 0.5 McFarland turbidity standard. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 52199 were used as control strains.

Molecular detection of *nuc, mec* and *van* genes:

The molecular analysis was carried out

in the Molecular Laboratory of BUTH. DNA was extracted from 200 µL of pelleted *S. aureus* isolates using the Quick-DNA™ Miniprep Kit (Lot 221058, Cat 4060). The extracted DNA was stored at 4°C until use. PCR amplification was performed in a 5× concentrated ready-to-use solution containing FIREPol® DNA polymerase, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.4 M Tris-HCl, 0.1 M $(NH_4)_2$, and 0.1% w/v Tween-20. Each 25µL reaction mixture contained 0.5µL forward primer, 0.5µL reverse primer, 9µL nuclease-free water, and 5µL template DNA. Primer sequences, amplicon sizes, and annealing temperatures are shown in Table 1.

Thermal cycling conditions were optimi zed for each gene. For *vanA,* initial denaturation at 96°C for 180 seconds, 35 cycles of 94°C for 45 seconds, 46°C for 45 seconds, and 72°C for 60 seconds, and final extension at 72°C for 360 seconds. For *vanB*; the same protocol with annealing at 50°C. For *mecA*; same protocol with annealing at 54°C. For the *nuc* gene; 37 cycles of 94°C for 60 seconds, 55°C for 30 seconds, and 72°C for 90 seconds, and final extension at 72°C for 210 seconds.

Positive controls included *E. faecium* ATCC 51559 (*vanA*), *E. faecalis* ATCC 51299 (*vanB*), and *S. aureus* ATCC 43300 (*mecA*). Negative controls (no template DNA) were included in each PCR run. PCR products (5 µL) were resolved on 1.5% agarose gels in 1× TBE buffer (pH 8.3) at 150 V for 2 hours. Gels were stained with SafeView® dye and visualised under ultraviolet light (Table 1).

Data analysis:

Data were cleaned and coded in Microsoft Excel and analysed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics summarised demographic and clinical data. Associations between categorical variables (resistance gene carriage and demographic/ clinical variables) were tested using the Chi-square test. A *p*-value less than 0.05 was considered statistically significant.

Results:

Prevalence of *nuc, mecA* and *vanA* genes in *S. aureus* isolates:

Of the total of 400 participants enrolled, 27 *S. aureus* isolates were recovered and confirmed by *nuc* gene amplification (Fig 1), giving 6.8% *S. aureus* nasal carriage rate in the study. *mecA* gene was detected in 25 of the 27 isolates (92.6%) (Fig 2), representing a 6.3% MRSA prevalence among the study participants. *vanA* gene was detected in 7 isolates (25.9% of isolates) (Fig 3), representing VRSA prevalence of 1.8% among the study participants. No isolate carried *vanB* gene (Fig 4).

Table 1: Primer sequences, amplicon sizes and PCR conditions

Gene	Primer sequence (5′→3′)	Amplicon size (bp)	Annealing temp (°C)	Positive control
mecA	F: GTAGAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	310	54	*S. aureus* ATCC 43300
vanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732	46	*E. faecium* ATCC 51559
vanB	F: GTGACAAACCGGAGGCGAGGA R: CCGCCATCCTCCTGCAAAAAA	647	50	*E. faecalis* ATCC 51299
Nuc	F: GCGATTGATGGTGATACGGTI R: AGCCAAGCCTTGACGAACTAAAGC	279	55	*S. aureus* (species control)

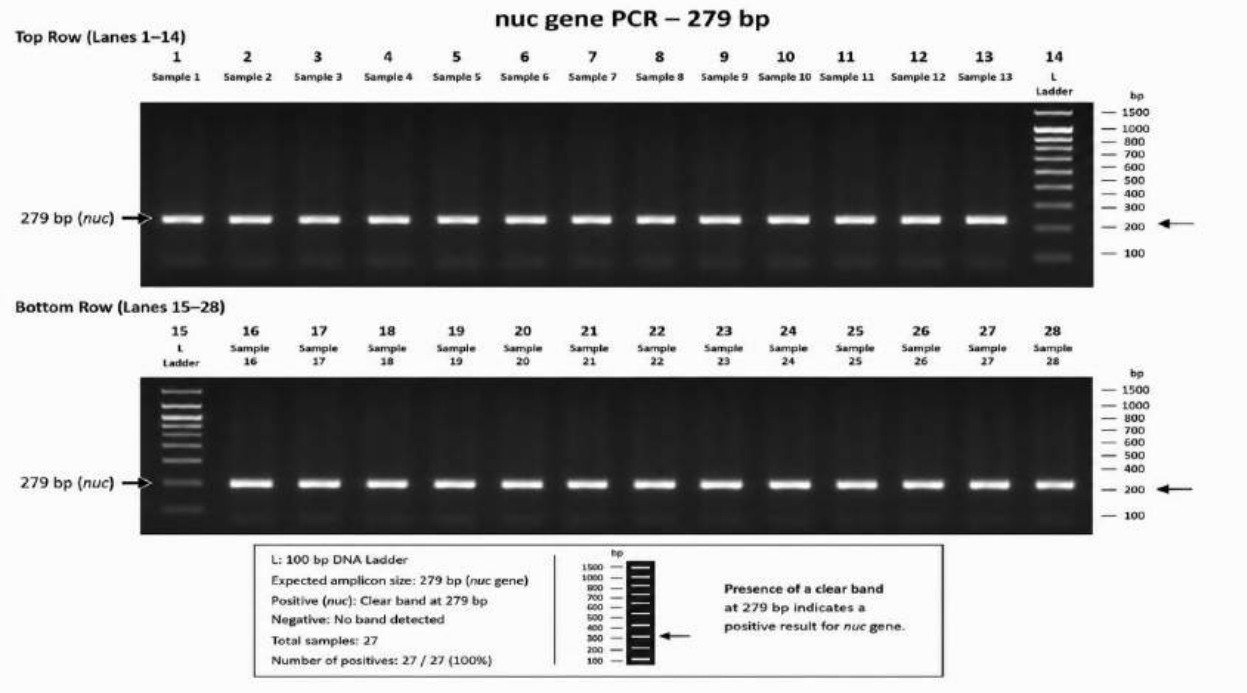

Fig. 1: Gel electrophoresis of PCR products (*nuc* gene at 279 bp)

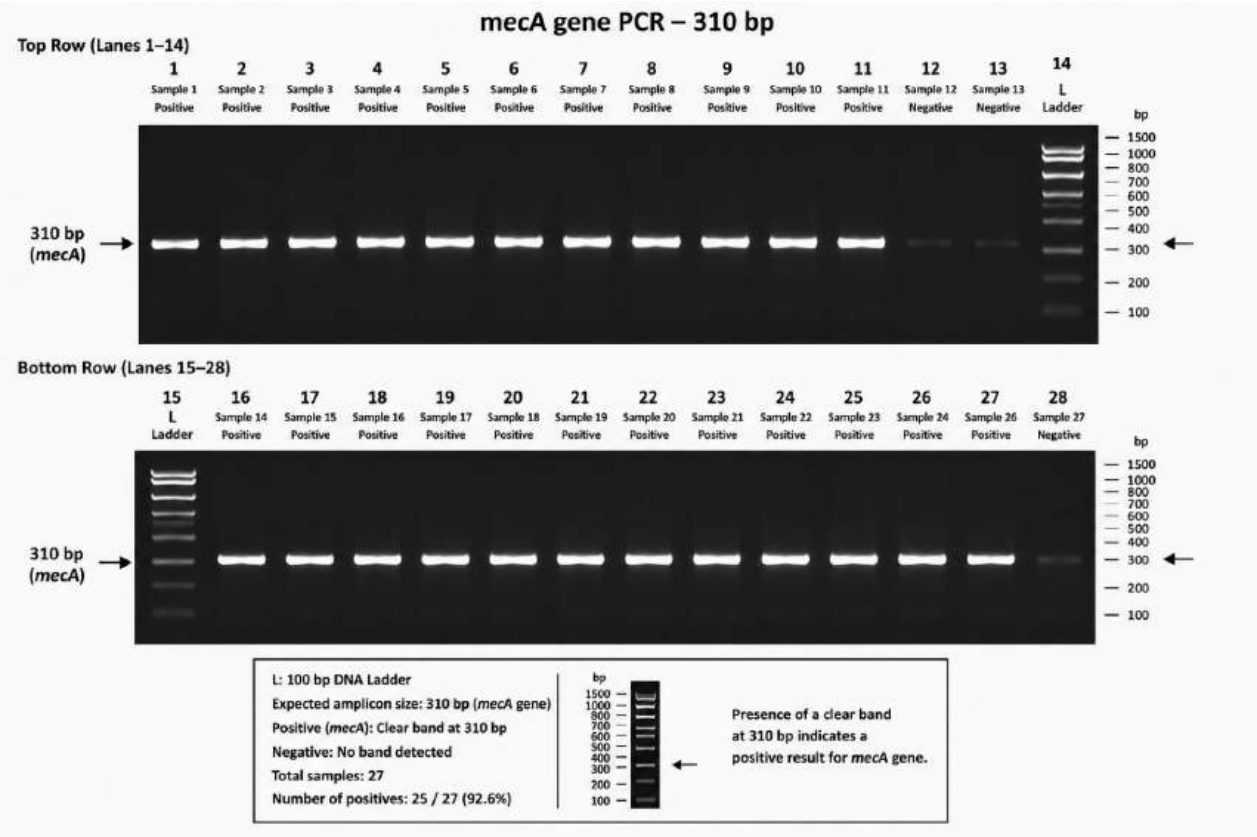

Fig 2: Gel electrophoresis of PCR products (*mecA* gene at 310 bp)

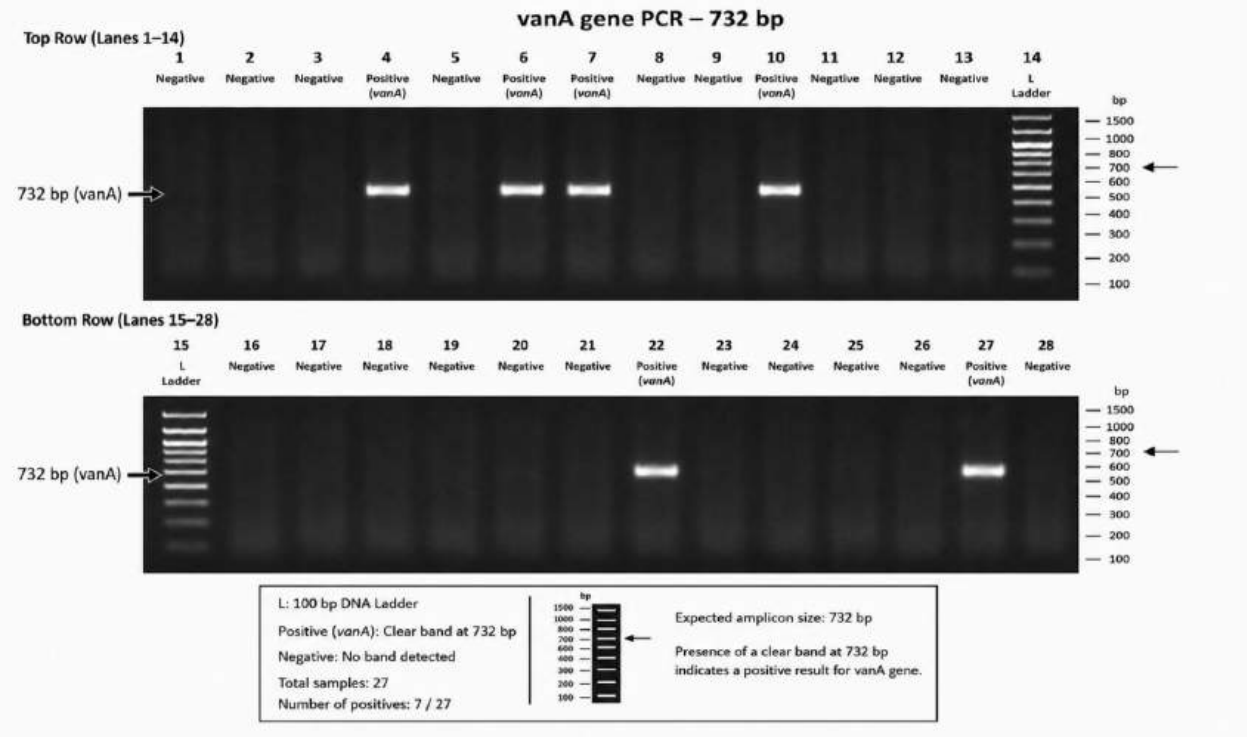

Fig 3: Gel electrophoresis of PCR products (*vanA* gene at 732 bp)

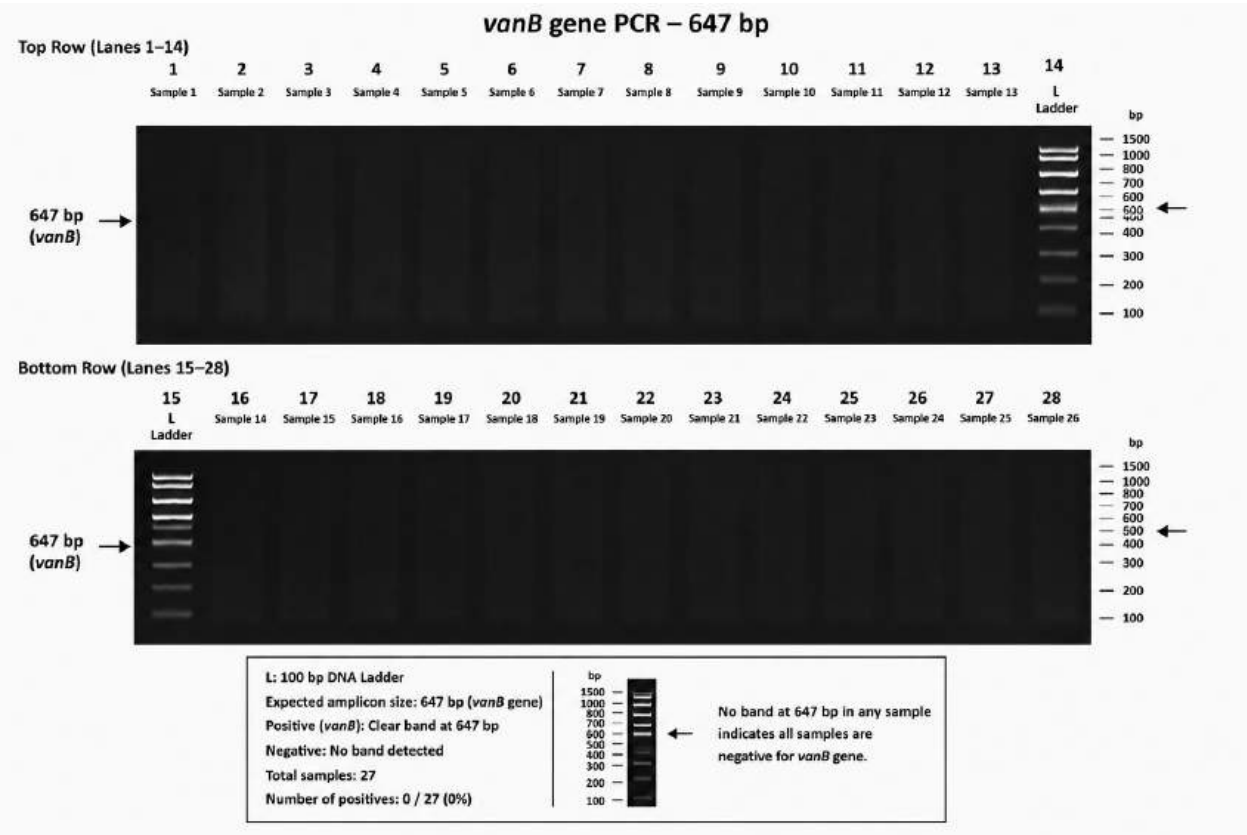

Fig 4: Gel electrophoresis of PCR products (*vanB* gene not amplified at expected 647 bp)

Association between resistance gene carriage and demographic/clinical variables of participants with *S. aureus* nasal colonization:

The highest frequency of vanA-positive *S. aureus* isolates was observed among participants aged 18–35 years (30.0%, n=3/10 isolates). However, age was not significantly associated with *vanA* carriage (χ^2=0.878, *p*=0.928). Similarly, gender, occupation, marital status, educational level, prior antibiotic use, ward type, and chronic medical conditions showed no significant associations with *vanA* carriage (Tables 2a and 2b).

Table 2a: Association between *vanA* gene carriage and demographic characteristics of participants with *Staphylococcus aureus* nasal colonization

Variable	*vanA*-positive (n, %)	Total *S. aureus* (n)	χ^2	df	*p*-value
Age group (years)					
5–17	1 (33.3)	3	0.878	4	0.928
18–35	3 (30.0)	10			
36–45	2 (25.0)	8			
46–65	1 (25.0)	4			
>65	0	2			
Gender					
Female	5 (22.7)	22	0.686	1	0.407
Male	2 (40.0)	5			
Occupation					
Unskilled	2 (33.3)	6	0.530	2	0.767
Semi-skilled	0	1			
Skilled	5 (25.0)	20			
Marital status					
Single	3 (37.5)	8	1.037	2	0.596
Married	4 (22.2)	18			
Widowed	0	1			
Education level					
No formal education	0	1	0.791	3	0.852
Primary	0	1			
Secondary	3 (30.0)	10			
Tertiary	4 (26.7)	15			

Table 2b: Association between *vanA* gene carriage and clinical characteristics of participants with *Staphylococcus aureus* nasal colonization

Variable	*vanA*-positive (n, %)	Total *S. aureus* (n)	χ^2	df	*p*-value
Antibiotic use (past 4 months)					
Yes	5 (23.8)	21	0.032	1	0.858
No	2 (33.3)	6			
Ward type					
Accident & Emergency	1 (50.0)	2	4.319	7	0.742
Paediatric	1 (50.0)	2			
Female Medical	1 (20.0)	5			
Male Medical	0 (0.0)	1			
Female Surgical	1 (20.0)	5			
Male Surgical	1 (50.0)	2			
ICU	0 (0.0)	1			
Obstetric	2 (22.2)	9			
Chronic medical conditions					
Yes	4 (25.0)	16	0.048	1	0.827
No	3 (27.3)	11			
Total	**7 (25.9)**	**27**			

All *S. aureus* isolates (100%) from participants aged 5–17 years, 18–35 years, 46–65 years, and >65 years were *mecA*-positive, while 75.0% of isolates from the 36–45 years age group carried the gene but age was not significantly associated with *mecA* carriage (χ^2= 5.130, p=0.274). Gender, occupation, marital status, educational level, prior antibiotic use, and chronic medical conditions also showed no significant associations (Tables 3a and 3b).

Ward type demonstrated a statistically significant association with *mecA* carriage (χ^2= 15.336, p=0.032). All *S. aureus* (100.0%) were *mecA* positive from accident and emergency (A & E), paediatric, female medical, male medical, male surgical, and obstetric wards. The female surgical ward had 80.0% (4 of 5) of the *S. aureus* positive for *mecA* while no *mecA*-positive isolate was detected in the ICU, although there was only one patient in the ICU at the time of the study (Table 3b).

Table 3a: Association between *mecA* gene carriage and demographic characteristics of participants with *Staphylococcus aureus* nasal colonization

Variable	*mecA*-positive (n, %)	Total *S. aureus* (n)	χ^2	df	p-value
Age group (years)					
5–17	3 (100.0)	3	5.130	4	0.274
18–35	10 (100.0)	10			
36–45	6 (75.0)	8			
46–65	4 (100.0)	4			
>65	2 (100.0)	2			
Gender					
Female	20 (90.9)	22	0.491	1	0.484
Male	5 (100.0)	5			
Occupation					
Unskilled	6 (100.0)	6	0.756	2	0.685
Semi-skilled	1 (100.0)	1			
Skilled	18 (90.0)	20			
Marital status					
Single	8 (100.0)	8	1.080	2	0.583
Married	16 (88.9)	18			
Widowed	1 (100.0)	1			
Education level					
No formal education	1 (100.0)	1	1.728	3	0.631
Primary	1 (100.0)	1			
Secondary	10 (100.0)	10			
Tertiary	13 (86.7)	15			

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Table 3b: Association between *mecA* gene carriage and clinical characteristics of participants with *Staphylococcus aureus* nasal colonization

Variable	*mecA*-positive n (%)	Total *S. aureus* (n)	χ^2	df	*p*-value
Antibiotic use (past 4 months)					
Yes	19 (90.5)	21	0.617	1	0.432
No	6 (100.0)	6			
Ward type					
Accident & Emergency	2 (100.0)	2	15.336	7	0.032*
Paediatric	2 (100.0)	2			
Female Medical	5 (100.0)	5			
Male Medical	1 (100.0)	1			
Female Surgical	4 (80.0)	5			
Male Surgical	2 (100.0)	2			
ICU	0 (0.0)	1			
Obstetric	9 (100.0)	9			
Chronic medical conditions					
Yes	14 (87.5)	16	1.485	1	0.223
No	11 (100.0)	11			
Total	**25 (92.6)**	**27**			

*Statistically significant at $p < 0.05$

Table 4: Antimicrobial susceptibility of *Staphylococcus aureus* nasal isolates

Antibiotic (disk content)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Cefoxitin (30 µg) – MRSA	17 (63.0)	0	10 (37.0)
Teicoplanin (30 µg)	25 (92.6)	0	2 (7.4)
Ceftaroline (30 µg)	27 (100)	0	0
Gentamicin (10 µg)	17 (63.0)	0	10 (37.0)
Erythromycin (15 µg)	15 (55.6)	0	12 (44.4)
Tetracycline (30 µg)	19 (70.4)	0	8 (29.6)
Levofloxacin (5 µg)	10 (37.0)	0	17 (63.0)
Clindamycin (2 µg)	23 (85.2)	1 (3.7)	3 (11.1)
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	23 (85.2)	4 (14.8)	0
Quinupristin–dalfopristin (15 µg)	27 (100)	0	0
Linezolid (30 µg)	27 (100)	0	0
Tedizolid (2 µg)	27 (100)	0	0
Telavancin (E-test) – VRSA	20 (74.1)	0	7 (25.9)

Antimicrobial susceptibility profile:

All 27 isolates (100.0%) were susceptible to ceftaroline, quinupristin–dalfopristin, linezolid, and tedizolid. Teicoplanin also demonstrated high activity (92.6% of isolates susceptible) as well as clindamycin and trimethoprim-sulfamethoxazole (with 85.2% isolates susceptibility to each). Resistance rates were highest for levofloxacin (63.0%), followed by erythromycin (44.4%), gentamicin (37.0%), cefoxitin (37%), and tetracycline (29.6%). Resistance to telavancin, suggestive of a VRSA phenotype, was detected in 7 of 27 isolates (25.9%) (Table 4).

Discussion:

Globally, *S. aureus* remains a significant pathogen, with increasing resistance to methicillin and vancomycin posing serious public health threats (3,4). Our study recorded a *mecA* prevalence of 92.6% among *S. aureus* isolates (prevalence of 6.3% among the study participants) is similar to reports from Cameroon (35) and previous Nigerian studies that reported 70–94% (36-38).

Regarding *vanA*, global participant-level carriage among *S. aureus*-colonised individuals typically ranges from 0.1–0.5% (18-41). National surveillance programmes in Europe and North America report *vanA*-positive *S. aureus* as exceedingly rare, with annual incidence rates below 0.01% (42,22). Our finding of 1.8% participant-level *vanA* carriage is close to findings in some African countries, signalling a potential public health emergency (43,44). A Kenyan tertiary hospital study reported participant level *vanA* carriage of 1.4% among *S. aureus* carriers (34,45), and a multicentre sub-Saharan African meta-analysis yielded a pooled prevalence of 0.4-1.0% (32,46). Our 1.8% rate exceeds these averages and suggests potential regional variations for emergence of glycopeptide resistance (47,48).

Although a statistically significant association was observed between *mecA* carriage and ward type (p=0.032), this finding should be interpreted with caution. The apparent significance may have been influenced by the ICU category, which included only one patient and no *mecA*-positive isolate. Such a small sample size may generate a potentially misleading association. Indeed, *mecA*-positive MRSA prevalence was consistently high across the remaining wards (80–100%). This finding mirrors studies from South Africa and Tanzania, where certain hospital units were identified as MRSA colonisation hotspots (49,50). Potential reasons for this include shared healthcare workers, inadequate hand hygiene practices, environmental persistence of resistant strains, and high rate of antibiotic use in specific units.

The absence of a statistically significant association between recent antibiotic use and resistance gene carriage should also be interpreted cautiously. While antibiotic exposure is a well-established driver of antimicrobial resistance (44), the specific antibiotics used by patients were not documented in this study. Consequently, some patients may not have been exposed to antibiotics that exert selective pressure for the emergence or persistence of MRSA. This lack of detailed antibiotic exposure data may have obscured a true relationship between antibiotic use and resistance gene carriage. In addition, the relatively small sample size and potential recall bias may have further limited the ability to detect such an association.

The lack of association with demographic variables is consistent with some previous studies (31), although it contrasts with findings from others (34). All isolates remained fully susceptible to linezolid, tedizolid, ceftaroline, and quinupristin-dalfopristin, consistent with other African studies (51,52). However, access and affordability of these antibiotics remain major concerns in Nigeria's public healthcare sector (53). Resistance to levofloxacin (63%), erythromycin (44%), and gentamicin (37%) severely limits empirical treatment options.

Beyond the absence of confirmatory gene sequencing, this study has several limitations. The sample size (n=27) is small, restricting generalisability. Only nasal swabs were analysed, excluding other sources. Clonal typing was not performed, limiting insights into transmission dynamics. Key risk factors such as prior hospitalisation, surgical history, length of stay, and invasive device use were not assessed. Healthcare workers and environmental reservoirs were not screened. The cross-sectional design prevents determination of causality.

Pending confirmation of *vanA* findings by sequencing, the results should not change clinical practice. If confirmed, they would represent a public health emergency requiring immediate IPC interventions. Priority actions include; (i) securing funding for sequencing of biobanked isolates; (ii) sequencing of all *vanA*-positive isolates; (iii) establishing a reference laboratory confirmation pathway for VRSA in Nigeria; and (iv) conducting a larger, multi-centre study.

Even without confirmation of the *vanA* gene findings, the high prevalence of *mecA* and its significant clustering in most wards of the hospital warrant action. Routine screening for MRSA colonisation on admission to high-risk wards, decolonisation using mupirocin, contact isolation precautions, and enhanced environmental disinfection should be considered (46).

Conclusion:

This study reports high participant-level *mecA* (6.3%) and *vanA* (1.8%) nasal carriage rates among hospitalized patients in BUTH Ilishan-Remo, Nigeria. However, these findings require independent confirmation (e. g. next-generation sequencing) before they can be accepted as valid. The biobanked isolates provide a valuable resource for this next step. The high *mecA* carriage in *S. aureus* isolates from inpatients across the major wards of the hospital suggests possible nosocomial transmission of MRSA, warranting further investigation using molecular typing. We caution against over-interpretation of the *vanA* data and recommend confirmatory studies as the immediate priority.

Contributions of authors:

OHN conceived and designed the study, participated in data collection, performed data analysis and interpretation, drafted the manuscript; OII contributed to the study design, supervised the research, critically reviewed the manuscript for intellectual content, and approved the final version; OTO contributed to data interpretation, manuscript review and revision, and approved the final version; ECJ contributed to data analysis and interpretation, reviewed the manuscript, and approved the final version; and AKB contributed to the study design, supervised the research, critically reviewed the manuscript for intellectual content, and approved the final version.

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Conflict of interest:

No conflict of interest is declared

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Original Article **Open Access**

Prevalence and distribution of the World Health Organization-Bacterial Priority Pathogens in selected hospitals in Katsina State, Nigeria

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Abstract:

Background: Infections caused by antibiotic-resistant bacteria have become a major global public health challenge, as they can reduce the effectiveness of modern medical treatments and clinical care. The most important of these pathogens are those listed in the World Health Organization (WHO) Bacterial Priority Pathogens List (BPPL) of 2024. This study determined the prevalence, distribution, and antimicrobial resistance patterns of the WHO bacterial priority pathogens in three selected hospital settings in Katsina State, Nigeria.
Methodology: We conducted a descriptive cross-sectional targeted survey of the WHO bacterial priority pathogens in 230 clinical samples from patients with suspected bacterial infections. Bacterial isolation and identification were performed using standard microbiological procedures. The resistance patterns were determined after performing antimicrobial susceptibility testing on the isolates in accordance with the 2024 CLSI guidelines. Further characterization of the pathogens was done using phenotypic resistance detection methods, including the double disk synergy test (DDST), Carba NP, and MRSA detection tests.
Results: Of the 230 samples, 113 samples (49.1%) yielded bacterial growth, with Gram-negative bacteria accounting for 58.4% of the isolates. The most frequently isolated bacterium was *Klebsiella aerogenes* (22.1%), followed by *Escherichia coli* (20.4%). The highest frequency of isolates (29.2%) was obtained from urine samples. The isolated bacteria showed high resistance to most antibiotics tested, with resistance rates to imipenem and cephalexin ranging from 78.3% to 100%, however gentamicin against Gram-negative and vancomycin against Gram-positive isolates were relatively effective. The overall prevalence of multidrug resistance (MDR) was 91.8% and the multiple antibiotic resistance (MAR) index value of all the isolates was greater than 0.2, which indicated exposure to high-risk sources of antimicrobial pressure. The WHO priority bacterial pathogens accounted for 77.0% of the total isolates. The most prevalent were third-generation cephalosporin-resistant Enterobacteriaceae (39.8%).
Conclusion: The WHO bacterial priority pathogens with high antimicrobial resistance are present in hospitals in Katsina State, Nigeria. These findings highlight the necessity of enhancing laboratory diagnostic capacity, routine culture and sensitivity testing before antibiotic administration, implementing effective antimicrobial stewardship programs, establishing regulations limiting over-the-counter antibiotic sales, and strengthening infection control and prevention practices within healthcare facilities in the State.

Keywords: Antimicrobial resistance, WHO, Bacterial priority, Multidrug-resistant bacteria, Carbapenem-resistance

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Prévalence et répartition des agents pathogènes bactériens prioritaires de l'Organisation Mondiale de la Santé dans certains hôpitaux de l'État de Katsina, au Nigéria

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Résumé:

Contexte: Les infections causées par des bactéries résistantes aux antibiotiques constituent un enjeu majeur de santé publique mondiale, car elles peuvent réduire l'efficacité des traitements médicaux modernes et des soins cliniques. Les pathogènes les plus importants sont ceux répertoriés dans la Liste des pathogènes bactériens prioritaires (LPBP) de l'Organisation mondiale de la Santé (OMS) de 2024. Cette étude a déterminé la prévalence, la distribution et les profils de résistance aux antimicrobiens des pathogènes bactériens prioritaires de l'OMS dans trois établissements hospitaliers sélectionnés de l'État de Katsina, au Nigéria.
Méthodologie: Nous avons mené une étude descriptive transversale ciblée sur les pathogènes bactériens prioritaires de l'OMS dans 230 échantillons cliniques provenant de patients présentant une suspicion d'infection bactérienne. L'isolement et l'identification des bactéries ont été réalisés selon les procédures microbiologiques standard. Les profils de résistance ont été déterminés après réalisation de tests de sensibilité aux antimicrobiens sur les isolats, conformément aux directives CLSI de 2024. Une caractérisation plus poussée des pathogènes a été effectuée à l'aide de méthodes de détection phénotypique de la résistance, notamment le test de synergie à double disque (TSDD), le test Carba NP et le test de détection du SARM.
Résultats: Sur les 230 échantillons, 113 (49,1%) ont présenté une croissance bactérienne, les bactéries Gram négatif représentant 58,4% des isolats. La bactérie la plus fréquemment isolée est *Klebsiella aerogenes* (22,1%), suivie d'*Escherichia coli* (20,4%). La plus grande proportion d'isolats (29,2%) a été obtenue à partir d'échantillons d'urine. Les bactéries isolées présentaient une forte résistance à la plupart des antibiotiques testés, avec des taux de résistance à l'imipénem et à la céphalexine allant de 78,3% à 100%. En revanche, la gentamicine contre les isolats Gram négatif et la vancomycine contre les isolats Gram positif se sont révélées relativement efficaces. La prévalence globale de la multirésistance (MDR) était de 91,8% et l'indice de résistance à de multiples antibiotiques (MAR) de tous les isolats était supérieur à 0,2, ce qui indique une exposition à des sources de pression antimicrobienne à haut risque. Les pathogènes bactériens prioritaires de l'OMS représentaient 77,0% du total des isolats. Les plus prévalents étaient les entérobactéries résistantes aux céphalosporines de troisième génération (39,8%).
Conclusion: Les pathogènes bactériens prioritaires de l'OMS présentant une forte résistance aux antimicrobiens sont présents dans les hôpitaux de l'État de Katsina, au Nigéria. Ces résultats soulignent la nécessité de renforcer les capacités de diagnostic en laboratoire, de réaliser systématiquement des cultures et des antibiogrammes avant l'administration d'antibiotiques, de mettre en œuvre des programmes efficaces de bon usage des antibiotiques, d'établir une réglementation limitant la vente d'antibiotiques sans ordonnance et de renforcer les pratiques de prévention et de contrôle des infections au sein des établissements de santé de l'État.

Mots-clés: Résistance aux antimicrobiens, OMS, bactéries prioritaires, bactéries multirésistantes, résistance aux carbapénèmes

Introduction:

The rising prevalence of bacterial infections that no longer respond to commonly used antibiotics has become a major public health issue worldwide. Antimicrobial resistance (AMR) threatens the efficacy of current treatment options and complicates the management of infectious diseases in both community and hospital settings (1). The spread of resistant microorganisms has not only resulted in longer hospital stays but also in increased mortality and treatment costs.

Poor hygiene practices, lack of awareness of infection prevention and control, improper use of antibiotics, and insufficient surveillance systems to monitor antimicrobial resistance are among the contributing factors to the high burden of antibiotic-resistant infections in Nigeria (2). Antibiotics are often administered in most healthcare institutions without laboratory confirmation of susceptibility against the causative pathogens and this situation only increases the prevalence of resistant strains (3).

In response to the growing global challenge of AMR, the WHO released an updated Bacterial Priority Pathogens List (BPPL) in May 2024. This list highlights bacterial species that pose the most serious risk to human health and is intended to support research, strengthen surveillance, and the development of new antimicrobial agents. The updated list includes 24 bacterial pathogens across 15 families and classifies them into three priority levels; critical, high, and medium. The classification of these pathogens is based on several criteria, which include severity of infections caused, mortality rate, disease burden, transmissibility, resistance trends, availability of effective treatment

options, and preventability in healthcare and community settings. Other considerations include the availability of new antibiotics currently under development and potential public health impact of the pathogens (2,4,5).

Pathogens classified in the critical priority group are carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant Enterobacterales (CRE), third-generation cephalosporin-resistant Enterobacterales, and rifampicin-resistant *Mycobacterium tuberculosis.* The high-priority group comprises fluoroquinolone-resistant *Salmonella* spp and *Shigella* spp, third-generation cephalosporin-resistant *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, and vancomycin-resistant *Enterococcus faecalis*. These pathogens are usually associated with healthcare-associated infections and are very difficult to treat. The medium-priority group includes macrolide-resistant Group A *Streptococcus*, macrolide-resistant *Streptococcus pneumoniae*, penicillin-resistant Group B *Streptococcus*, and ampicillin-resistant *Haemophilus influenzae* (2).

Globally, it has been estimated that approximately 700,000 deaths occur annually as a result of drug-resistant infections, largely associated with the WHO-BPPL, and if effective interventions are not implemented, this number could rise to about 10 million deaths each year by 2050, with nearly 4.1 million deaths expected to occur in Africa alone. In addition to its effects on health, AMR will result in significant global economic losses (6,7).

In Nigeria, particularly in Katsina State, there is limited empirical data or research evidence on the epidemiology of the WHO bacterial priority pathogens. Various studies have indicated that their epidemiology is poorly documented, particularly in hospital settings (8). Such limited empirical data prevent the healthcare provider from taking effective steps to control infections and restrict the ability of government to develop specific interventions in the sphere of public health. WHO has also highlighted that region-specific data on the epidemiology of these pathogens are important for understanding the burden of disease, planning effective infection prevention and control activities, guiding antimicrobial stewardship, and supporting the formulation of disease-specific treatment guidelines (7).

Like many other States in Nigeria, Katsina faces growing issues with AMR and hospital-acquired infections. The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have led to increased morbidity, mortality, and health care expenditures. Poor antibiotic use, poor infection control practices, and limited diagnostic capacity are among the factors that still contribute to the spread of resistant bacteria in healthcare facilities (3,9,10).

The inadequate comprehensive data on these pathogens complicate the development of effective interventions and policies to limit their effects and transmission. It is therefore necessary to provide credible information on their prevalence, distribution, and resistance patterns to enhance infection control practices and standard patient management. This study, by providing information on the prevalence and distribution of the WHO bacterial priority pathogens in selected hospitals in Katsina State, contributes to the initiative in understanding the local burden of AMR. The results will be useful for decision-making, antimicrobial stewardship programs, and infection prevention and control practices in healthcare facilities in the State. Finally, this research will contribute to national and global strategies to reduce the impact of drug-resistant infections.

Materials and method:

Study design and setting:

The study was a hospital-based descriptive cross-sectional survey in three selected hospitals located in Katsina State, Nigeria. The three hospitals were Federal Teaching Hospital Katsina, General Hospital Kurfi, and General Hospital Dutsinma.

Study participants and selection:

Patient recruitment and sample collection were carried out within different clinical units of these hospitals, including accident and emergency units, medical and surgical wards, pediatric wards, laboratory units, and other relevant hospital departments. Participants included in the study consisted of patients of all age groups with suspected bacterial infections, regardless of gender, who were not on any antibiotic therapy or had not received antibiotics in the preceding two weeks before the study. Participants who were on antibiotic therapy or had received antibiotics in the 2 weeks preceding the study, and those with other medical conditions other than bacterial infections were excluded.

Ethical considerations:

The study was conducted after obtaining ethical approval from the ethical committee chairman at each of the selected State hospitals and from the Ministry of Health in Katsina State,

with ethical approval number MOH/ADM/SUB/1152/1/1064. Ethical approval was also obtained from the Federal Teaching Hospital, Katsina, with the assigned number FTHKHRECDEG24/06/22C/248. Strict confidentiality was maintained throughout the study.

Sample size determination:

The sample size for the study was determined using the standard sample size estimation formula for cross-sectional study design. The calculation was based on the previously reported prevalence rate of bacterial infections of 28.3% in a study done by Mouanga-Ndzime et al., (11) and the calculated sample size was increased by 10% to account for attrition due to contaminated specimens, non-response, or missing data, resulting in an adjusted sample size of 230.

Sample collection and processing:

The clinical specimens collected from enrolled participants included blood, urine, stool, vaginal and wound swabs, using standard aseptic techniques. The specimens were appropriately labeled, and transported to the laboratory in accordance with stipulated guidelines.

Isolation and identification of bacterial pathogens:

To isolate the bacteria, clinical samples were inoculated on the appropriate culture media that included Blood agar, MacConkey agar, Cetrimide agar, and Nutrient agar using sterile procedures. The inoculated plates were incubated aerobically at 37°C for 24 hours. The morphological characteristics of bacterial colonies were observed for colony size, shape, margin, elevation, color, and haemolytic patterns (12).

Gram staining was performed to identify Gram-positive and Gram-negative bacteria. Additional identification was performed using different biochemical tests as appropriate, including catalase, coagulase, motility, indole production, urease activity, citrate utilization, oxidase test, methyl red test, Vogue-Proskauer test, bile solubility, optochin and bacitracin susceptibility.

The procedures were performed in accordance with the microbiological guidelines outlined by Cheesbrough (12). Distinct colonies were sub-cultured to isolate pure cultures, which were stored for later use in bacterial identification and antimicrobial susceptibility testing.

Antimicrobial susceptibility testing of isolates:

The Kirby-Bauer disk diffusion antimicrobial susceptibility test (AST) was performed on Mueller-Hinton (MH) agar plate. Pure bacterial colonies were resuspended in 5 ml of sterile saline, and the suspension was adjusted to a turbidity of 0.5 McFarland standard. A sterilized swab was then used to spread the standardized culture over the surface of MH agar plates to ensure uniform growth of bacterial colonies. The isolated bacteria were inoculated onto plates containing standard antibiotic discs including cephalosporin (ceftriaxone 30µg, cephalexin 30 µg), carbapenem (meropenem 10µg, imipenem 10µg), fluoroquinolone (ciprofloxacin 5µg), aminoglycoside (gentamicin 15µg), and cotrimoxazole (10µg), with penicillin (amoxicillin-clavulanic acid (20/10µg), glycopeptide (vancomycin 30µg) and macrolide (azithromycin 15µg) for Gram-positive isolates only.

The plates were incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition around each antibiotic disc were measured in millimeters, and the results interpreted as susceptible, intermediate, or resistant based on the Clinical and Laboratory Standards Institute (CLSI) guideline (13).

Determination of multi-drug resistance (MDR) and multiple antibiotic resistance index (MARI):

Multi-drug resistance (MDR) among the bacterial isolates was determined using the criteria set by the Centers for Disease Control and Prevention, which is resistance of bacterial isolates to at least one antibiotic in three or more antibiotic classes (8). In addition, the MAR index was also calculated for each isolate using the formula; MAR index = number of antibiotics to which the isolate is resistant, divided by the total number of antibiotics tested. MAR index values greater than 0.2 were deemed to represent isolates with a background of high antibiotic exposure and high AMR selection pressure (14).

Double-disc synergy test for detection of ESBL production:

The double-disc synergy test (DDST), as previously described by Jarlier et al., (15) and in accordance with the recommendations of CLSI was used to detect ESBL production among the Gram-negative isolates. Cefotaxime (30µg), ceftriaxone (30µg), and ceftazidime (30µg) discs were placed 30 mm apart from an amoxicillin-clavulanate (30µg) disc, and Gram-negative bacterial suspension standardized to a 0.5 McFarland turbidity was inoculated onto MH agar plate. The zone expansion or keyhole effect between the cephalosporin and amoxicillin-clavulanate discs observed 24 hours after incuba-

tion at 37°C was used to confirm the production of ESBL (15).

Carba NP test for detection of carbapenemase production:

A rapid colorimetric assay, known as the Carba NP Test, was used to detect carbapenemase production among the Gram-negative isolates. In this method, intracellular enzymes were released after bacterial cells were lysed, and the lysate was then mixed with a solution containing imipenem and the pH indicator phenol red. A color change from red to yellow indicated antibiotic hydrolysis and confirmed carbapenemase production (16).

Detection of fluoroquinolone resistance in *Salmonella* isolates:

Resistance to fluoroquinolones in the *Salmonella* isolates was detected using the disc diffusion method. The suspension was incubated for 18-24 hours at 35°C, and a smaller inhibition zone around the ciprofloxacin disc meant reduced susceptibility or resistance (13, 17).

Detection of methicillin resistance in *Staphylococcus aureus* isolates:

Methicillin resistance in *S. aureus* isolates was detected by uniformly inoculating MH agar plate with a standardized suspension (0.5 McFarland) of overnight culture of *S. aureus* isolates and overlying the surface with a 30 µg cefoxitin disc. The plate was then incubated for 18-24 hours at 35°C, and the diameter of the inhibitory zone was measured. Methicillin-resistant *S. aureus* (MRSA) was identified when the inhibition zone was 21 mm or less, while a zone of 22 mm or more indicated a methicillin-susceptible strain (13).

Detection of vancomycin resistance in *Enterococcus faecalis* isolates:

Vancomycin resistance in *Enterococcus faecalis* was determined by standard AST in accordance with the CLSI guidelines. A bacterial suspension that has been standardized to 0.5 McFarland turbidity was spread out uniformly on the Mueller-Hinton agar plates. On the inoculated agar surface, a vancomycin disc (30 µg) was placed thereafter. An incubation period of 18-24 hours at 37°C was observed on the plates, and the diameter of the inhibition zone surrounding the vancomycin disc was measured and interpreted according to CLSI criteria. Strains that had lower susceptibility or resistance to vancomycin were considered vancomycin-resistant *Enterococcus* (VRE) (13,18).

Results:

Distribution of bacterial isolates in clinical specimens:

Of the 230 clinical samples collected from patients in the three selected hospitals, 113 samples (49.1%) grew bacteria. Of these, Gram-negative bacteria accounted for 66 isolates (58.4%) and Gram-positive bacteria for 47 isolates (41.6%) (Fig 1).

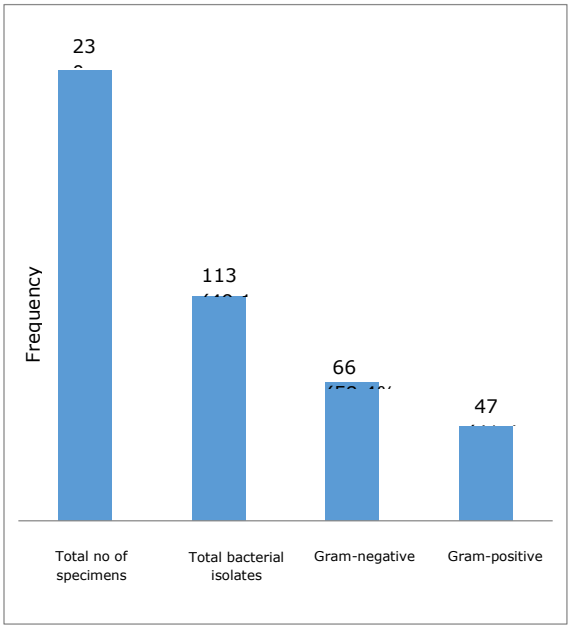

Fig 1: Distribution of bacterial isolates from clinical specimens in selected hospitals in Katsina State, Nigeria

Frequency of bacterial species from clinical specimens of participants:

Of the total of 113 bacterial isolates, the most frequent is *Klebsiella (Enterobacter) aerogenes* with 25 isolates (22.1%), followed by *Escherichia coli*, which accounted for 23 isolates (20.4%). *Proteus vulgaris* was also frequently isolated (10.6%, n=12) and was among the major pathogens identified during the study. Conversely, only one isolate (0.9%) of *A. baumannii* was identified (Table 1).

Table 1: Frequency of bacterial species from selected hospitals in Katsina State, Nigeria

Bacterial pathogen	Number (%)
Klebsiella (Enterobacter) aerogenes	25 (22.1)
Escherichia coli	23 (20.4)
Streptococcus species	15 (13.3)
Klebsiella pneumoniae	13 (11.5)
Proteus vulgaris	12(10.6)
Enterococcus faecalis	11 (9.7)
Staphylococcus aureus	9 (7.9)
Salmonella species	2 (1.7)
Pseudomonas aeruginosa	2 (1.7)
Acinetobacter baumannii	1 (0.9)
Total	**113 (100.0)**

Distribution of bacterial isolates according to specimen types:

The distribution of bacterial species also varied according to specimen type. In blood samples, *K. pneumoniae* was the most common pathogen detected accounting for 6 isolates (5.3%), and *A. baumannii* appeared less frequently. In urine samples, *E. coli* was the most common organism with 13 isolates (11.6%) followed by *Streptococcus* species with 2 isolates (1.8%). In stool samples, *E. aerogenes* had the highest number of isolates (n=17, 15.0%), whereas *E. faecalis* had the fewest (n=3, 2.7%). The most common isolates in wound swab were *Streptococcus* species, with 8 isolates (7.1%), followed by *E. aerogenes* with the least number of 1 isolate (0.9%). Only *Streptococcus* species were recovered from vaginal swab samples as shown in Table 2.

Antibiotic resistance profile of bacterial isolates:

The antibiotic resistance patterns of the bacterial isolates are summarized in Table 3. The analysis showed that there was a high resistance of the isolates to various antibiotics used. It is worth noting that very high rates of resistance to imipenem and cephalexin of between 88% and 100% against the bacterial species isolated was recorded. *Salmonella* species as well as *Pseudomonas aeruginosa* were totally resistant to at least seven out of the ten evaluated antibiotics. Similarly, *S. aureus* was 100% resistant to co-trimoxazole, meropenem, and imipenem.

Gentamicin and vancomycin, however, were highly effective against most of the Gram-negative and Gram-positive bacterial isolates respectively. There was no resistance to gentamicin among *K. aerogenes* while there was also no resistance to vancomycin by *Streptococcus* species, *E. faecalis* and only 14.3% of *S. aureus* were resistant to it. Comparatively low resistance was also observed for most of the isolates to azithromycin except *E. faecalis* (36.4%), *Salmonella* spp (40.0%), *Proteus vulgaris* (66.7%) and *P. aeruginosa* (100.0%).

Table 2: Frequency distribution of bacterial species by specimen types in selected hospitals in Katsina State, Nigeria

Clinical specimens	Bacterial isolates	Number positive (%)
Blood	*Escherichia coli*	5 (4.4)
	Streptococcus spp	3 (2.7)
	Klebsiella pneumoniae	6 (5.3)
	Proteus vulgaris	2 (1.8)
	Enterococcus faecalis	4 (3.5)
	Staphylococcus aureus	4 (3.5)
	Salmonella spp	2 (1.8)
	Acinetobacter baumannii	1 (0.9)
Urine	*Enterobacter aerogenes*	7 (6.2)
	Escherichia coli	13 (11.5)
	Streptococcus spp	2 (1.8)
	Klebsiella pneumoniae	3 (2.7)
	Proteus vulgaris	4 (3.5)
	Enterococcus faecalis	4 (3.5)
Stool	*Enterobacter aerogenes*	17 (15.0)
	Escherichia coli	5 (4.4)
	Klebsiella pneumoniae	4 (3.5)
	Enterococcus faecalis	3 (2.7)
Wound swab	*Enterobacter aerogenes*	1 (0.9)
	Streptococcus spp	8 (7.1)
	Proteus vulgaris	6 (5.3)
	Staphylococcus aureus.	5 (4.4)
	Pseudomonas aeruginosa	2 (1.8)
Vaginal swab	*Streptococcus* spp	2 (1.8)
Total		**113 (100.0)**

Table 3: Antibiotic resistance pattern of bacterial pathogens from clinical samples in three selected hospitals in Katsina State, Nigeria

Pathogen/Antibiotics	**Number of resistant isolates (%)**									
	AMX	**AZM**	**CFM**	**LEX**	**CIP**	**COT**	**GEN**	**IMP**	**MEM**	**VAN**
Escherichia coli (n=23)	5 (21.7)	1 (4.3)	9 (39.1)	20 (86.9)	10 (43.5)	18 (78.3)	1 (4.3)	22 (95.6)	18 78.3)	NA
Enterobacter aerogenes (n=25)	8 (32.0)	1 (4.0)	12 (48.0)	22 (88.0)	3 (12.0)	20 (80.0)	0	25 (100)	21(84.0)	NA
Streptococcus spp (n=15)	3 (25.0)	2 (15.4)	6 (43.8)	13 (87.5)	1 (12.5)	10 (68.8)	1 (6.3)	14 (93.8)	7 (50.0)	0
Klebsiella pneumoniae (n=13)	3 (23.1)	2 (8.3)	7 (58.3)	12 (92.3)	2 (15.4)	8 (61.5)	5 (38.5)	13 (100)	11(84.6)	NA
Enterococcus faecalis (n=11)	4 (36.4)	4 (36.4)	6 (54.5)	11 (100)	3 (27.3)	10 (90.9)	5 (45.5)	9 (81.8)	9 (81.8)	0
Proteus vulgaris (n=12)	8 (66.7)	8 (66.7)	10 (83.3)	11 (91.7)	6 (50.0)	10 (83.3)	6 (50.0)	12 (100)	11(91.7)	NA
Staphylococcus aureus (n=9)	8 (85.7)	1 (14.3)	8 (85.7)	8 (85.7)	8 (85.7)	9 (100)	0	9 (100)	9 (100)	1 (14.3)
Salmonella spp (n=2)	2 (100)	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	NA
Pseudomonas aeruginosa (n=2)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	NA
Acinetobacter baumannii (n=1)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	NA

AMX: Amoxicillin, AZM: Azithromycin, CFM: Cefixime, LEX: Cephalexin, CIP: Ciprofloxacin, COT: Co-trimoxazole, GEN: Gentamicin, IMP: Imipenem, MEM: Meropenem, VAN: Vancomycin. NA: Not applicable

Antibiotic resistance phenotypes of the bacterial isolates:

The resistance phenotypes of the bacterial isolates obtained from clinical samples are presented in Table 4. *Salmonella* species*, P. aeruginosa and S. aureus* were the isolates with highest resistance phenotypes (resistant to antibiotics in 6 classes), while *Streptococcus* spp on the other hand, had the least resistance phenotypes (resistant to two antibiotic classes). Generally, the results showed that approximately 90% of Gram-positive bacterial isolates were susceptible to vancomycin and Gram-negative bacteria to gentamicin, indicating that these antibiotics could still be viable treatment options for bacterial infections in the study area.

Table 4: Antibiotic resistant phenotypes of bacterial isolates from clinical samples in three selected hospitals in Katsina State, Nigeria

Bacterial isolate	**Resistant phenotype**	**No of antibiotic class**		**Number of isolates (%)**	**Effective antibiotics (based on susceptibility)**
Escherichia coli	AMX, CFX/LEX, CIP, COT, IMI/MEM	5	MDR	21 (91.3)	AZM, GEN
Klebsiella pneumoniae	AMX, CFX/LEX, COT, IMI/MEM	4	MDR	11 (84.6)	AZM, CIP, GEN
Enterobacter aerogenes	AMX, CFX/LEX, IMI/ MEM	3	MDR	22 (88.0)	AZM, CIP, COT, GEN
Proteus vulgaris	AMX, CFX/LEX, COT, IMI/MEM	4	MDR	11 (92.0)	AZM, CIP, GEN
Salmonella species	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	2 (100.0)	GEN
Acinetobacter baumannii	AMX, CFX/LEX, CIP, COT, IMI/MEM	5	MDR	1 (100.0)	AZM, GEN
Pseudomonas aeruginosa	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	2 (100.0)	GEN
Staphylococcus aureus	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	9 (100.0)	GEN, VAN
Enterococcus faecalis	CFX/ LEX, COT, IMI/MEM	3	MDR	9 (81.8)	AMX, AZM, CIP, GEN, VAN
Streptococcus species	LEX, IMI/MEM	2	Not MDR	12 (80.0)	AMX, AZM, CIP, COT, GEN, VAN

AMX: Amoxicillin, AZM: Azithromycin, CFM: Cefixime, LEX: Cephalexin, CIP: Ciprofloxacin, COT: Co-trimoxazole, GEN: Gentamicin, IMP: Imipenem, MEM: Meropenem, VAN: Vancomycin. MDR: Resistance to ≥3 antibiotics from different antimicrobial classes

Multidrug resistance patterns of isolates:

Of 113 bacterial isolates, 100 (91.8%) were resistant to three or more antibiotic classes, thereby meeting the definition of multidrug resistance (MDR). MDR was most frequently observed in S. aureus (100.0%, n=9/9), Salmonella species (100%, n=2/2), P. aeruginosa (100.0%, n=2/2) and A. baumannii (100.0%, n=1/1). It is noteworthy that all these bacterial pathogens are among the WHO bacterial priority pathogens (Table 4).

Multiple antibiotic resistance index of isolates:

The term MAR index gives insight into the contamination risk in the selected hospitals as well as level of antibiotic pressure. All isolates showed MAR index values exceeding the high-risk threshold of 0.2 (Table 5). Salmonella spp had the highest MAR index (0.85), while K. pneumoniae had the lowest (0.40). The mean MAR index of the isolates is 0.594 (Table 5).

Table 5: Multiple antibiotic resistance (MAR) indices of bacterial pathogens from clinical samples in three selected hospitals in Katsina State, Nigeria

Bacterial pathogen	Number of isolates	No of antibiotic tested	MAR index
Salmonella species	2	10	0.85
Pseudomonas aeruginosa	2	10	0.80
Acinetobacter baumannii	1	10	0.70
Staphylococcus aureus	9	10	0.67
Proteus vulgaris	12	10	0.57
Enterococcus faecalis	11	10	0.56
Escherichia coli	23	10	0.51
Klebsiella (Enterobacter) aerogenes	15	10	0.45
Streptococcus species	15	10	0.43
Klebsiella pneumoniae	13	10	0.40

MAR Index = Number of antibiotics to which isolate is resistant divided by the total number of antibiotics tested

Table 6: Distribution of WHO Bacterial Priority Pathogens isolated from three selected health facilities in Katsina State, Nigeria

Priority bacterial pathogen	Number	Percentage
3rd generation cephalosporin-resistant Enterobacteriaceae	45	39.8
Carbapenem-resistant Enterobacteriaceae	23	20.4
Methicillin-resistant *Staphylococcus aureus* (MRSA)	9	8.0
Macrolide-resistant *Streptococcus* spp	4	3.5
Fluoroquinolone-resistant *Salmonella* spp	2	1.8
Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA)	2	1.8
Penicillin-resistant *Streptococcus* spp	1	0.9
Carbapenem-resistant *Acinetobacter baumannii* (CRAB)	1	0.9
Non-priority bacterial pathogens	26	23.0
Total	**113**	**100.0**

Frequency distribution of the WHO bacterial priority pathogens:

The distribution of WHO bacterial priority pathogens identified in the study is presented in Table 6. The third-generation cephalosporin-resistant Enterobacteriaceae was the most commonly identified priority pathogen with 45 isolates (39.8%), followed by carbapenem-resistant Enterobacteriaceae with 23 isolates (20.4%). Also, 9 isolates (8.0%) were MRSA. Ampicillin-resistant *Enterococcus faecalis* and carbapenem resistant *A. baumannii* (CRAB) were the least common priority pathogens (one isolate of each, 0.9%). Twenty-six isolates (23.0%) were not listed in the WHO priority list.

According to the WHO 2024 classification of bacteria pathogens based on priority, the critical priority group constituted the largest proportion of isolates in this study, accounting for 69 isolates (79.3%), and the lowest frequency was medium priority group, with 5 isolates (5.7%) (Table 6).

Discussion:

The detection of bacterial growth in 113 of 230 clinical specimens (49.1%) in this study indicates a significant burden of bacterial infections among patients visiting the selected hospitals in Katsina State, Nigeria. Other hospital-based studies have reported similar culture positivity rates in Nigeria, indicating that bacterial infections remain widespread in healthcare facilities across most low- and middle-income countries (19,20). These findings underscore the importance of routine laboratory diagnosis in the successful management of infectious diseases.

The results also indicated that Gram-negative bacteria were more commonly isolated than Gram-positive bacteria, a trend that aligns with results from several studies conducted in Nigeria and other African countries, where Gram negative bacteria pathogens were identified as the major causes of both community- and hospital-acquired infections (21,22). The high prevalence of Gram-negative bacteria isolates can be partially attributed to their ability to survive in hospital settings and to a variety of mechanisms that allow them to resist antimicrobial agents.

Enterobacter aerogenes (formerly known as *Klebsiella aerogenes)* was the most common bacterial species identified in the study, closely followed by *E. coli.* These pathogens are widely recognized as important causes of urinary tract infections, bloodstream infections, and gastrointestinal diseases. The high occurrence of *E. coli* in urine samples observed in this study is consistent with previous reports from Nigerian hospitals, where *E. coli* has been identified as the predominant pathogen associated with urinary tract infections (19,23). Although the occurrence of *A. baumannii* was lower, it is a clinically important pathogen due to its classification as a critical priority pathogen and known to cause severe hospital-acquired infections associated with very high mortality rates (24,25).

A microanalysis of the specimen types indicated that the largest number of bacterial isolates was obtained from urine samples, implying that urinary tract infections are a common clinical problem in the study area. The same has been reported in other studies conducted in Nigerian hospitals, where the highest frequency of bacterial isolates was consistently detected in urine samples (26). Several isolates were also recovered from stool samples, which could indicate the enteric infection burden in the area. Poor sanitation and water quality might be the causes of this trend.

The results of blood culture showed that the most common organisms associated with blood infections were *K. pneumoniae* and *E. coli*. These pathogens have often been involved in sepsis, and associated with high morbidity and mortality, especially in hospitalized patients (22). The isolation of these bacteria from blood samples underscores the severity of bacterial infections encountered in the healthcare facilities included in this study.

The antimicrobial susceptibility test results showed high levels of resistance to antibiotics such as cephalexin, imipenem and meropenem. The high resistance to carbapenems is especially alarming because carbapenems are commonly viewed as the 'last-resort' treatment options for infections caused by multidrug-resistant organisms (25,27). These levels of resistance indicate that there are increasingly more obstacles to the treatment of severe bacterial infections in the study areas. The high resistance levels observed in this study are comparable to findings reported in other regions of Nigeria, where inappropriate use of antibiotics is a primary driver of antimicrobial resistance (26,28). Self-medication, over-the-counter availability of antibiotics, and empirical treatments are likely contributing factors to the high resistance levels observed.

Although resistance levels to several antibiotics by the isolates were high, resistance to gentamicin and vancomycin (by Gram-positive bacteria) were relatively low, which implies that these antibiotics may still be effective against certain infections in the study area. Similar results have been reported in prior Nigerian res-

earch, in which gentamicin retained significant activity against both Gram-positive and Gram-negative pathogens (21). Nonetheless, continued use of a few effective antibiotics may ultimately lead to increased resistance if antimicrobial stewardship programs are not established. Another important finding of this study was the high MAR index values observed among the isolates, all greater than 0.2. These values are typically regarded as indicators of bacterial strains that have evolved in environments with high antibiotic exposure. This has also been observed in hospitals, where antibiotic misuse and overuse contribute to intense selection on microbial populations (19).

The overall prevalence of MDR in the study was notably high. Several bacterial species, including *S. aureus*, *Salmonella* species, *P. aeruginosa* and *A. baumannii*, were 100% MDR. This observation is consistent with findings from earlier Nigerian studies that reported high rates of MDR among bacterial pathogens categorized as WHO priority organisms (22,29). The presence of MDR bacterial pathogens poses a serious challenge to clinical management because it significantly reduces available treatment options and may lead to prolonged hospital stays and increased treatment costs. Consistent with the study objective of identifying WHO bacterial priority pathogens, a large proportion of the isolates detected belonged to the critical priority category, particularly third-generation cephalosporin-resistant and carbapenem-resistant Enterobacteriaceae. These are also consistent with the reports provided at the global and national level that consider Enterobacteriaceae among the key factors that increase the burden of AMR (2,30).

Moreover, the detection of MRSA in the study confirms earlier findings that MRSA infections are very common in healthcare centers in Nigeria (29). The presence of pathogens belonging to the medium priority category was less common, but they also pose a significant risk of disease and, especially, can be detrimental to vulnerable groups, like children, older people, as well as immunocompromised patients (31).

There are some limitations in this study. First, this study was conducted in only three hospitals in Katsina State, Nigeria, therefore, the findings may not fully represent the situation in all healthcare facilities within the State or Nigeria as a whole. In addition, bacterial identification and resistance detection were based mainly on phenotypic laboratory methods, and molecular techniques were not used to confirm carriage of resistance genes by the isolates.

Conclusion:

The findings of this study indicate that WHO priority bacterial pathogens are common in hospital settings in Katsina State, Nigeria. Gram-negative bacteria were the most frequent pathogens identified, with *K. aerogenes* and *E. coli* being the most common bacterial species from the clinical samples tested.

The study also showed that most bacterial isolates exhibited high resistance, with some resistant to antibiotics used as last-line treatments for serious bacterial infections. Moreover, high frequency of the isolates was MDR, underscoring the increasing difficulty of treating bacterial infections in the study area. These results underline the necessity to intensify antimicrobial stewardship initiatives, routine laboratory diagnosis and infection prevention and control measures in healthcare facilities to contain the proliferation of resistant bacterial pathogens.

Contributions of authors:

SHA, MGL and HMR were involved in the study conceptualization, data collection, laboratory analysis, data interpretation, manuscript drafting and critical revision of the manuscript. All authors approved the final version submitted for publication.

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Conflicts of interest:

Authors declare no conflicts of interest.

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Original Article **Open Access**

Comparative analysis of biofilm formation in carbapenem-resistant and carbapenem-susceptible *Klebsiella pneumoniae* clinical isolates from a tertiary hospital in Nigeria

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Abstract:

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infections pose a major challenge due to limited therapeutic options. Biofilm formation has been proposed as a potential contributor to antimicrobial resistance; however, evidence regarding its association with carbapenem resistance in *K. pneumoniae* remains conflicting. Notably, data from Nigeria are scarce. The objective of this study is to compare biofilm formation between carbapenem-resistant and carbapenem susceptible *K. pneumoniae* (CRKP and CSKP) isolated from blood samples.
Methodology: This comparative laboratory-based study analysed 46 stored blood culture isolates of *K. pneumoniae* from a tertiary hospital in Nigeria. Re-identification was done using VITEK® 2 automated system and carbapenem susceptibility testing was carried out using Kirby-Bauer disc diffusion method. Two methods of biofilm formation assessment; Congo Red Agar (CRA) and microtiter plate assay, were used. Statistical analysis was done using Fisher Exact test, with Odds ratio and 95% confidence interval, and $p<0.05$ considered statistical significance. For statistical analysis of biofilm strength determined by the microtiter plate assay, isolates were dichotomised into strong and non-strong biofilm producers.
Results: A total of 46 *K. pneumoniae* blood isolates were studied, of which 30 (65.2%) were CSKP and 16 (34.8%) were CRKP. Using the CRA method, 16 isolates (34.8%) were biofilm-positive, while 30 (65.2%) were negative; 6 of 16 (37.5%) CRKP and 10 of 30 (33.3%) CSKP isolates were biofilm formers, but the difference was not statistically significant (OR=1.200; 95% CI=0.3385-4.254; $p=1.000$). Similarly, the microtiter plate assay identified 42 isolates (91.3%) as strong biofilm producers and 4 (8.7%) as non-strong biofilm producers; 14 of 16 (87.5%) CRKP and 28 of 30 (93.3%) CSKP isolates were strong biofilm producers, but the difference was also not statistically significant (OR=0.500; 95% CI=0.06355-3.934; $p=0.6019$).
Conclusion: Our study found no significant association between carbapenem resistance and biofilm formation, suggesting that biofilm formation may act as an independent bacterial survival strategy rather than a direct contributor to carbapenem resistance.

Keywords: *Klebsiella pneumoniae*, Biofilm formation, Carbapenem resistance, Congo Red Agar, Microtiter plate assay

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Analyse comparative de la formation de biofilm chez des isolats cliniques de Klebsiella pneumoniae résistants et sensibles aux carbapénèmes provenant d'un hôpital universitaire du Nigéria

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Résumé:

Contexte: Les infections à *Klebsiella pneumoniae* résistante aux carbapénèmes (CRKP) constituent un défi majeur en raison du nombre limité d'options thérapeutiques. La formation de biofilm a été proposée comme facteur potentiel de résistance aux antimicrobiens; cependant, les données concernant son association avec la résistance aux carbapénèmes chez *K. pneumoniae* restent contradictoires. Notamment, les données nigérianes sont rares. L'objectif de cette étude est de comparer la formation de biofilm entre les souches de *K. pneumoniae* résistantes et sensibles aux carbapénèmes (CRKP et CSKP) isolées à partir d'hémocultures.
Méthodologie: Cette étude comparative en laboratoire a analysé 46 isolats de *K. pneumoniae* provenant d'hémocultures conservées d'un hôpital de référence au Nigéria. La réidentification a été effectuée à l'aide du système automatisé VITEK® 2 et les tests de sensibilité aux carbapénèmes ont été réalisés par la méthode de diffusion sur disque de Kirby-Bauer. Deux méthodes d'évaluation de la formation de biofilm ont été utilisées: la gélose au rouge Congo (CRA) et le test sur microplaque. L'analyse statistique a été réalisée à l'aide du test exact de Fisher, avec calcul du rapport de cotes et de l'intervalle de confiance à 95%, et un seuil de signification statistique fixé à $p<0,05$. Pour l'analyse statistique de la force du biofilm déterminée par le test sur microplaque, les isolats ont été classés en deux groupes: producteurs forts et faibles de biofilm.
Résultats: Au total, 46 isolats sanguins de *K. pneumoniae* ont été étudiés, dont 30 (65,2%) étaient des CSKP et 16 (34,8%) des CRKP. Par la méthode CRA, 16 isolats (34,8%) étaient positifs pour la formation de biofilm, tandis que 30 (65,2%) étaient négatifs; 6 des 16 isolats CRKP (37,5%) et 10 des 30 isolats CSKP (30,0 %) formaient un biofilm, mais la différence n'était pas statistiquement significative (OR=1,200; IC à 95%=0,3385-4,254; p=1,000). De même, le test sur microplaque a identifié 42 isolats (91,3%) comme producteurs forts de biofilm et 4 (8,7%) comme producteurs faibles de biofilm. Quatorze des seize isolats de CRKP (87,5%) et 28 des 30 isolats de CSKP (93,3%) étaient de forts producteurs de biofilm, mais la différence n'était pas statistiquement significative (OR=0,500; IC à 95%=0,06355-3,934; p=0,6019).
Conclusion: Notre étude n'a pas mis en évidence d'association significative entre la résistance aux carbapénèmes et la formation de biofilm, suggérant que cette dernière pourrait constituer une stratégie de survie bactérienne indépendante plutôt qu'un facteur direct de résistance aux carbapénèmes.

Mots-clés: *Klebsiella pneumoniae*, formation de biofilm, résistance aux carbapénèmes, gélose au rouge Congo, test sur microplaque

Introduction:

Infections caused by *Klebsiella pneumoniae* are becoming progressively more difficult to treat as antimicrobial resistance continues to expand at an alarming rate, especially resistance to carbapenems, which are often regarded as the last resort antibiotics for treatment of infections caused by multidrug-resistant (MDR) pathogens (1,2). Several international studies have documented a substantial occurrence in carbapenem-resistant *K. pneumoniae* (CRKP) over the years (3-5). Similarly, two systematic reviews have reported a rising trend of CRKP in Nigerian hospitals, with prevalence rates ranging from 20.2% to 26.3% (6,7).

This increasing trend is linked to increased death rates, extended hospital admissions and limited access to effective therapeutic options (8,9). Despite this, active surveillance of CRKP remains challenging in resource-limited settings due to financial constraints, which contributes to the under-recognition of the actual disease burden (10,11). In response to this growing threat, the World Health Organization (WHO) has identified CRKP as a priority for the development of novel therapeutic agents (12, 13).

Resistance to carbapenems largely arises from the acquisition of carbapenem-hydrolysing enzymes, notably KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lactamase) and OXA-48-type β-lactamases (14-16). These enzymes hydrolyse carbapenems and render them ineffective, often resulting in treatment failures and prolonged hospital stays (17).

Beyond enzyme-linked resistance, the persistence and virulence of *K. pneumoniae* infections are strongly influenced by the ability of the organism to develop biofilms, which are complex microbial assemblies encased within a self-generated extracellular matrix that attaches to both living tissues and inert surfaces (18, 19). This protective structure limits the effectiveness of antimicrobial therapy and impairs host immune clearance, thereby complicating eradication of infection (19). According to Makhrmash et al., *fimH*, *mrkA,* and *mrkD* genes, which encode fimbrial adhesion proteins that help the bacteria attach to surfaces and host cells, are commonly found in *K. pneumoniae* and are strongly linked to biofilm-producing isolates (20).

There are mixed findings on whether carbapenem resistance is linked to biofilm formation in *K. pneumoniae*. Some studies reported a significant association, suggesting that isolates resistant to carbapenems tend to form stronger biofilms (21,22). In contrast, Fang et al., (23) found that the regulatory gene *mrkH*, which plays a key role in biofilm development, is often absent in carbapenem-resistant strains, leading these isolates to produce weaker biofilms. Sabenca et al., (24) further showed that the presence of KPC enzymes does not necess-

arily correspond with increased biofilm-forming ability. Similarly, Cepas et al., (25) observed that multidrug-resistant strains were not more likely to form higher-level biofilms than non-multidrug-resistant strains (25). These studies indicate that although biofilm formation and carbapenem resistance may interact, the exact nature of this relationship is still unclear.

Several studies from Nigeria have reported the prevalence and antimicrobial resistance patterns of *K. pneumoniae*, with a primary focus on phenotypic resistance profiles and molecular characterisation (26-29). No study from Nigeria has specifically examined the association between carbapenem-resistant *K. pneumoniae* (CRKP) and carbapenem-suscepti ble *K. pneumoniae* (CSKP). Since enhanced biofilm formation can increase bacterial persistence and reduce treatment efficacy, this study aims to compare biofilm formation between CRKP and CSKP isolated from blood samples in a tertiary facility in Lagos and to determine if there is any relationship between biofilm formation and carbapenemase resistance.

Materials and method:

Study setting, design and ethical approval:

This was a comparative laboratory-based study carried out in the Department of Medical Microbiology and Parasitology Laboratory, Lagos University Teaching Hospital (LUTH). Ethical approval was obtained from the hospital's ethical committee with Health Research Committee Assigned Number: ADM/DSCST/HREC/APP/7759.

Sample size determination and collection of isolates:

Sample size was determined using a 5.2% prevalence of carbapenem-resistant *Klebsiella pneumoniae* at LUTH (30), with a 2:1 carbapenem-susceptible to carbapenem-resistant ratio to enhance statistical power. Based on this ratio, an estimated sample size of 30-45 isolates was calculated. Ultimately, 46 archived blood isolates (16 carbapenem-resistant and 30 carbapenem-susceptible) collected between January and August 2025 were included. These isolates were recovered from routinely stored glycerol stocks at -80°C in the Department of Medical Microbiology and Parasitology Laboratory, Lagos University Teaching Hospital (LUTH).

Re-identification of *K. pneumoniae:*

Stored isolates were first cultured on MacConkey agar and Blood agar and incubated at 37°C for 18–24 hours. On MacConkey agar, colonies were large, pink, and mucoid, reflecting lactose fermentation. On blood agar, colonies appeared large, greyish-white, smooth, and non-haemolytic. Colonies showing these characteristic features were further analysed and confirmed using the VITEK®2 automated system for species identification.

Carbapenem susceptibility testing:

Carbapenem susceptibility testing was done using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar, in accordance with 2025 Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem discs used were meropenem (10µg) and imipenem (10µg). Plates were incubated at 37°C for 18-24 hours. Inhibition zone diameters were measured and interpreted according to CLSI. Isolates with diameters ≥23mm were classified as CSKP, those between 20-22mm as intermediate, and ≤19mm as CRKP.

Biofilm detection:

Biofilm formation in the CRKP and CSKP isolates was assessed using two complementary methods; qualitative Congo Red Agar (CRA) assay and quantitative 96-well Microtiter Plate assay.

CRA assay:

The CRA method, described by Harika et al., (31) and Wilson et al., (32) was used as an initial qualitative screening technique. The assay is based on the principle that biofilm-producing bacteria synthesize extracellular polysaccharides capable of binding Congo red dye. When the dye interacts with the slime layer produced by the organism, it alters colony morphology. Biofilm-forming isolates typically appear as black colonies with a dry crystalline consistency, whereas non-producers appear red or pink. All CRA plates were independently interpreted by two observers to reduce subjectivity, and discrepancies were resolved by repeat testing. Duplicate testing was also performed to enhance result reliability.

Microtiter plate assay:

Although CRA provides a simple and rapid visual assessment, it is limited in its ability to distinguish between weak and moderate biofilm producers. Therefore, a standardized quantitative microtiter plate assay was employed for more accurate measurement of biofilm production strength. This method follows the principles described by Wilson et al., (32) and Stepanović et al., (33). Briefly, isolates were allowed to adhere and form biofilms in sterile 96-well micro titer plates for 24 hours. The resulting biofilm was stained with crystal violet, after which the

bound dye was solubilized with ethanol.

The optical density (OD) of each well was measured at 532–630 nm using a microplate reader. Four negative control wells containing only sterile broth were included. Their mean OD and standard deviation (SD) were calculated, and the optical density cut-off (ODc) was determined using the formula: ODc = mean OD of negative controls + (3×SD). For each isolate, the average OD from its duplicate wells was calculated and the ODc was subtracted to obtain the final OD value used for classification. Biofilm production was categorized as follows; No biofilm: OD ≤ ODc; Weak biofilm: ODc < OD ≤ 2×ODc; Moderate biofilm: 2×ODc < OD ≤ 4×ODc; and Strong biofilm: OD > 4×ODc

Although a standard positive control strain was not available during laboratory analysis, quality assurance was maintained through the use of negative control wells, strict adherence to established protocols and duplicate testing to ensure consistency and reproducibility of the results.

Statistical analysis:

The Fisher's Exact test was performed using IBM SPSS (34) to determine significant association between carbapenem resistance and biofilm formation, with Odds ratio and 95% confidence interval. Statistical significance was set at $p<0.05$. For the purpose of inferential analysis, the strength of biofilm production determined by the microtiter plate assay was dichotomized into two categories; strong biofilm producers and non-strong biofilm producers, with the latter comprising weak, moderate, and non-biofilm-producing isolates for analysis using the Fisher's Exact test.

Results:

Identification and distribution of isolates:

A total of 46 stored *Klebsiella pneumoniae* isolates obtained from blood cultures were identified using the VITEK®2 system. Of these, 30 (65.2%) were carbapenem-susceptible (CSKP), while 16 (34.8%) were carbapenem-resistant (CRKP).

Results of biofilm detection by Congo Red Agar assay:

Using the Congo Red Agar method, 16 (34.8%) of the 46 *K. pneumoniae* isolates produced black, dry crystalline colonies indicative of biofilm formation, while 30 (65.2%) produced pink colonies and were classified as non-biofilm producers.

Association between biofilm formation and carbapenem resistance by CRA assay

Biofilm formation was observed in both carbapenem-resistant and carbapenem-susceptible isolates. Six of 16 (37.5%) CRKP and 10 of 30 (33.3%) CSKP isolates were biofilm formers. However, Fisher's exact test showed no statistically significant association between carbapenem resistance and biofilm formation (OR= 1.200; 95% CI = 0.3385 - 4.254; p = 1.000) (Table 1).

Biofilm quantification by Microtiter Plate assay:

The distribution of isolates into non-biofilm, weak, moderate and strong biofilm producers is summarised in Table 2. The result of the microtiter plate assay is shown in Fig 1.

Table 1: Frequency of biofilm formation by qualitative Congo Red agar assay among carbapenem-resistant and carbapenem-susceptible *Klebsiella pneumoniae* blood isolates

Isolates	Biofilm positive (%)	Biofilm negative (%)	Total	OR (95% CI)	*p* value
Carbapenem-Resistant	6 (37.5)	10 (62.5)	16	1.200 (0.3385-4.254)	1.000
Carbapenem-Susceptible	10 (33.3)	20 (66.7)	30		
Total	**16 (34.8)**	**30 (65.2)**	**46**		

OR=Odds ratio; CI=Confidence interval

Table 2: Classification of biofilm production by *Klebsiella pneumoniae* isolates on Microtiter plate assay

Biofilm production category	Number of isolates	Percentage (%)
No biofilm	0	0
Weak biofilm	2	4.3
Moderate biofilm	2	4.3
Strong biofilm	42	91.4
Total	**46**	**100.0**

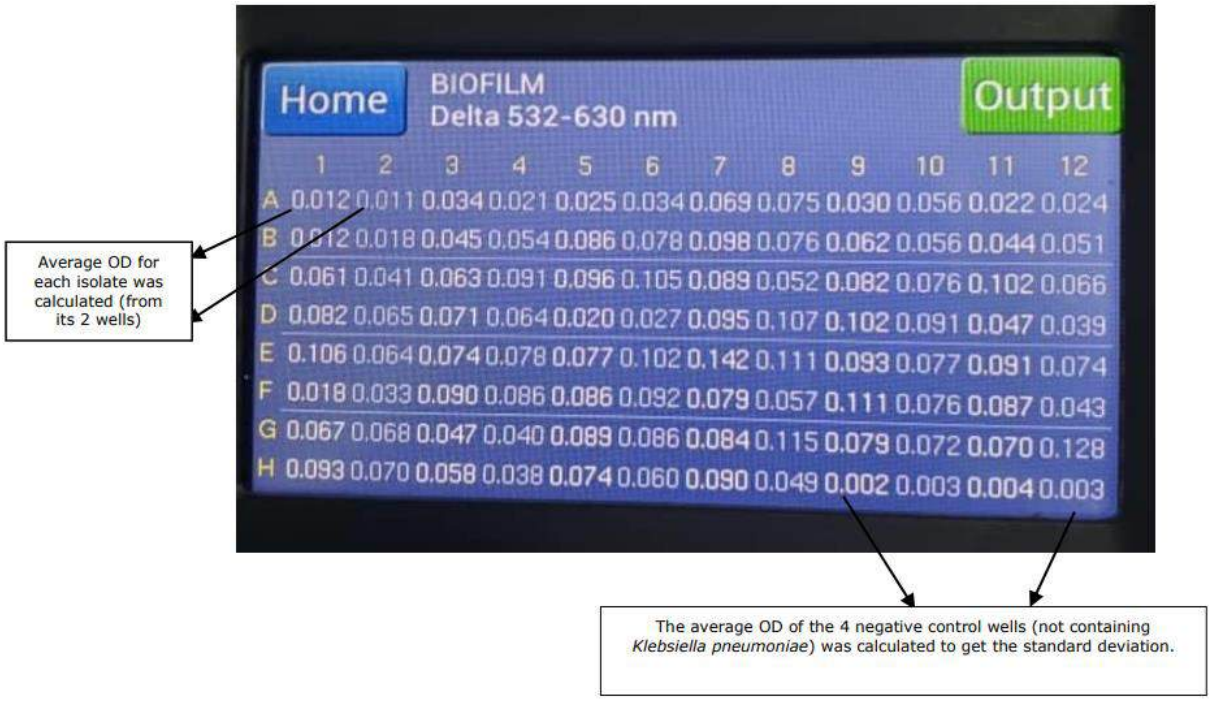

Fig 1: Result of Microtitre Plate assay for biofilm detection

Table 3: Frequency of biofilm formation by quantitative Microtitre Plate assay among carbapenem-resistance and carbapenem-susceptible *Klebsiella pneumoniae* blood isolates

Isolates	**Strong biofilm (%)**	**Non-strong biofilm (%)**	**Total**	**OR (95% CI)**	***p* value**
Carbapenem-Resistant	14 (87.5)	2 (12.5)	16	0.500 (0.0636-3.934)	0.6019
Carbapenem-Susceptible	28 (93.3)	2 (6.7)	30		
Total	**42 (91.3)**	**4 (8.7)**	**46**		

OR=Odds ratio; CI=Confidence interval

Association between biofilm strength and carbapenem resistance by Microtiter Plate assay:

When biofilm strength was analysed, majority of the carbapenem-resistant and carbapenem-susceptible isolates (91.3%) were classified as strong biofilm producers; 14 of 16 (87.5%) CRKP and 28 of 30 (93.3%) CSKP isolates were strong biofilm producers. Fisher's Exact test demonstrated no statistically significant association between carbapenem resistance and biofilm strength (OR=0.500; 95% CI= 0.06355-3.934; *p*=0.6019) (Table 3).

Discussion:

This study compared biofilm formation using both qualitative and quantitative phenotypic methods. The consistently higher detection of biofilm production by the microtiter plate (tissue culture plate) shows the greater sensitivity of quantitative approaches in capturing biofilm-forming capacity, irrespective of carbapenem susceptibility. In contrast, the Congo Red Agar method identified fewer biofilm-producing isolates, reflecting its reliance on subjective colour interpretation and limited ability to detect weaker or intermediate biofilm phenotypes. Similar discrepancies between CRA and microtiter-based assays have been reported elsewhere, reinforcing the view that CRA is best suited for preliminary screening rather than definitive assessment of biofilm formation (35-37).

The central finding of this study is the absence of an association between biofilm-forming capacity and carbapenem resistance in *K. pneumoniae*. This suggests that biofilm formation and carbapenem resistance may represent distinct adaptive strategies rather than interdependent phenotypes. Similar observations have been reported by Sabença et al., (24) and Bandeira et al., (38) who demonstrated that strong biofilm formation does not necessarily coincide with carbapenemase production or

high-level antimicrobial resistance. Collectively, these findings support the notion that carbapenem resistance in *K. pneumoniae* is more strongly driven by specific resistance mechanisms, such as carbapenemase production, porin alterations and efflux pump activity than by biofilm-forming ability alone (39).

Comparing these results with other studies shows a mixed pattern. For instance, Rahdar et al., (21) while correlating biofilm formation with carbapenem resistance, found a strong positive association between these two factors, with the majority of resistant isolates being strong biofilm producers. The researchers suggested that the biofilm formation may function as an adaptive mechanism increasing antibiotic resistance and, therefore, making such strains harder to treat. Jaisal et al., (22) also found an association between carbapenemase-producing *K. pneumoniae* and biofilm formation, showing that most of the isolates were moderate to strong biofilm producers. However, it did not compare biofilm formation between resistant and susceptible isolates. Contrarily, Fang et al., (23) identified that carbapenem-susceptible isolates were more likely to carry the *mrkH* gene, thus helping biofilm formation, and showed stronger biofilm formation compared with the resistant ones. The conflicting outcomes across studies might be related to differences in sample size, detection methods, and the local pattern of antimicrobial resistance.

From an infection-control perspective, the high prevalence of biofilm formation among both carbapenem-resistant and susceptible isolates has important clinical implications. Biofilm-producing strains can persist on medical devices, hospital surfaces, and indwelling catheters, facilitating recurrent or chronic infections even in the absence of overt antimicrobial resistance. Consequently, infection-prevention strategies should incorporate measures that directly target biofilm disruption, rather than focusing solely on antimicrobial susceptibility profiles (40). Moreover, biofilm-associated persistence may promote the maintenance and dissemination of resistance determinants, highlighting the need to address biofilm formation as an independent risk factor in healthcare-associated infections (41,42).

Conclusion:

This study found no evidence of an association between biofilm-forming capacity and carbapenem resistance in *K. pneumoniae*, indicating that biofilm formation may represent an independent bacterial survival strategy rather than a direct determinant of carbapenem resistance. The ability of both carbapenem-resistant and carbapenem-susceptible isolates to form strong biofilms underscores the clinical relevance of biofilm-mediated persistence in healthcare settings, irrespective of antimicrobial susceptibility profiles.

The findings should be interpreted in light of certain limitations, including the relatively small sample size, the absence of a standard positive control strain, and the exclusive reliance on phenotypic methods for biofilm detection. While these approaches are practical and widely used, they do not capture the molecular complexity underlying biofilm regulation and resistance mechanisms. Future studies incorporating larger isolate collections and molecular characterization of both carbapenem resistance determinants and biofilm-associated genes are warranted to better elucidate the interplay between resistance and virulence in *K. pneumoniae*.

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Contributions of authors:

The study was a collaborative effort, with all authors making substantive contributions to the manuscript. OBM led the experimental design and conducted the data analysis. AV provided overall supervision of the study, while ORO contributed through critical review and intellectual refinement of the manuscript.

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Authors declare no conflicts of interest.

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The role of 16S rRNA sequencing in bridging phenotypic–genotypic gaps in *Salmonella* identification: Evidence from a Nigerian endemic zone

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Abstract:

Background: Accurate laboratory identification of *Salmonella* species is essential for effective diagnosis, antimicrobial stewardship, and surveillance of enteric fever. Conventional culture and biochemical methods remain the primary diagnostic approach in many laboratories in resource-limited countries but are prone to misidentification because several members of the family Enterobacteriaceae exhibit similar phenotypic characteristics. This study evaluated the role of 16S rRNA gene sequencing in validating phenotypic identification of *Salmonella* isolates obtained from patients with suspected enteric fever in Nigeria.

Methodology: Blood samples collected from a total of 425 patients with suspected enteric fever were cultured using standard bacteriological procedures. Presumptive *Salmonella* isolates were identified by conventional biochemical tests, including triple sugar iron (TSI) agar reactions, urease, citrate, motility, and indole tests. Genomic DNA was extracted from all presumptive isolates, and bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) and sequenced by Sanger method. Sequence identities were determined using the National Center for Biotechnology Information (NCBI) BLAST database, while phylogenetic relationships were inferred using the Maximum Likelihood method with bootstrap analysis.

Results: Conventional phenotypic methods identified 32 (7.5%) isolates as *Salmonella* from the patients. However, sequencing confirmed only 25 (78.1% of presumptive isolates and 5.9% of blood cultures as *Salmonella*, while 7 (21.9%) were reclassified as non-*Salmonella* Enterobacteriaceae, including *Enterobacter oligotrophicus* (n=3), *Enterobacter hormaechei* (n=1), *Citrobacter freundii* (n=1), *Citrobacter youngae* (n=1), and *Klebsiella oxytoca* (n=1). Using sequencing as the reference standard, phenotypic identification demonstrated 100% sensitivity (95% CI: 84.6–100.0), 81.6% specificity (95% CI: 66.6–91.6), positive predictive value of 78.1% (95% CI: 60.0–90.7), and negative predictive value of 100% (95% CI: 88.8–100.0), with substantial agreement (Cohen's κ =0.78, p<0.001). Among the confirmed isolates, *S.* Typhimurium (n=11) was predominant, followed by untyped *S. enterica* (n=6), and single isolates of *S.* Enteritidis, *S.* Heidelberg, *S.* Infantis, *S.* Typhi, *S.* Livingstone, *S.* Paratyphi B, *S. diarizonae*, and *S. bongori*. Phylogenetic reconstruction grouped these isolates into four major clades with strong bootstrap support, confirming distinct evolutionary lineages within the circulating population.

Conclusion: Conventional phenotypic methods substantially overestimated the prevalence of *Salmonella* infection because of bacterial misidentification. Integration of 16S rRNA gene sequencing into diagnostic workflows significantly improved species confirmation, refined prevalence estimates, and provided valuable phylogenetic information that can strengthen antimicrobial resistance surveillance and public health decision-making in endemic settings.

Keywords: *Salmonella;* 16S rRNA; Sanger sequencing; Enteric fever; Blood culture; Phylogenetic analysis

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Rôle du séquençage de l'ARNr 16S dans la résolution des discordances phénotypiques et génotypiques pour l'identification de *Salmonella*: données issues d'une zone endémique du Nigéria

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Résumé:

Contexte: L'identification précise des espèces de *Salmonella* en laboratoire est essentielle pour un diagnostic efficace, une gestion optimale des antimicrobiens et la surveillance de la fièvre typhoïde. Les méthodes conventionnelles de culture et de biochimie restent la principale approche diagnostique dans de nombreux laboratoires des pays à ressources limitées, mais elles sont sujettes à des erreurs d'identification, car plusieurs membres de la famille des Enterobacteriaceae présentent des caractéristiques phénotypiques similaires. Cette étude a évalué le rôle du séquençage du gène de l'ARNr 16S dans la validation de l'identification phénotypique des isolats de *Salmonella* obtenus chez des patients présentant une suspicion de fièvre typhoïde au Nigéria.
Méthodologie: Des échantillons de sang prélevés chez un total de 425 patients présentant une suspicion de fièvre typhoïde ont été mis en culture selon les procédures bactériologiques standard. Les isolats présumés de Salmonella ont été identifiés par des tests biochimiques conventionnels, notamment les réactions sur gélose TSI (Triple Sugar Iron), les tests à l'uréase, au citrate, de mobilité et d'indole. L'ADN génomique a été extrait de tous les isolats présumés, et le gène de l'ARNr 16S bactérien a été amplifié par réaction en chaîne par polymérase (PCR) et séquencé par la méthode de Sanger. Les identités de séquences ont été déterminées à l'aide de la base de données BLAST du NCBI (Centre National d'Information sur la Biotechnologie), tandis que les relations phylogénétiques ont été inférées par la méthode du maximum de vraisemblance avec analyse de bootstrap.
Résultats: Les méthodes phénotypiques conventionnelles ont identifié 32 isolats (7,5%) comme étant des *Salmonella* chez les patients. Cependant, le séquençage n'a confirmé que 25 isolats (78,1% des isolats présomptifs et 5,9% des hémocultures) comme étant des *Salmonella*, tandis que 7 (21,9%) ont été reclassés comme Enterobacteriaceae non-*Salmonella*, notamment *Enterobacter oligotrophicus* (n=3), *Enterobacter hormaechei* (n=1), *Citrobacter freundii* (n=1), *Citrobacter youngae* (n=1) et *Klebsiella oxytoca* (n=1). En utilisant le séquençage comme méthode de référence, l'identification phénotypique a démontré une sensibilité de 100% (IC à 95%: 84,6–100,0), une spécificité de 81,6% (IC à 95%: 66,6–91,6), une valeur prédictive positive de 78,1% (IC à 95%: 60,0–90,7) et une valeur prédictive négative de 100% (IC à 95%: 88,8–100,0), avec une concordance substantielle (κ de Cohen=0,78, p<0,001). Parmi les isolats confirmés, *S*. Typhimurium (n=11) était prédominant, suivi de *S. enterica* non typé (n=6), et d'isolats uniques de *S.* Enteritidis, *S*. Heidelberg, *S*. Infantis, *S*. Typhi, *S*. Livingstone, *S*. Paratyphi B, *S. diarizonae* et *S. bongori*. La reconstruction phylogénétique a regroupé ces isolats en quatre clades principaux avec un fort soutien bootstrap, confirmant des lignées évolutives distinctes au sein de la population circulante. **Conclusion:** Les méthodes phénotypiques conventionnelles surestimaient considérablement la prévalence de l'infection à *Salmonella* en raison d'erreurs d'identification bactérienne. L'intégration du séquençage du gène de l'ARNr 16S dans les flux de travail diagnostiques a significativement amélioré la confirmation des espèces, affiné les estimations de prévalence et fourni des informations phylogénétiques précieuses qui peuvent renforcer la surveillance de la résistance aux antimicrobiens et la prise de décision en matière de santé publique contexte endémique.

Mots-clés: *Salmonella*; ARNr 16S; séquençage Sanger; fièvre typhoïde; hémoculture; analyse phylogénétique

Introduction:

Enteric fever remains a major public health challenge in many low- and middle-income countries, particularly in sub-Saharan Africa and South Asia, where inadequate sanitation, unsafe drinking water, rapid urbanization, and limited access to quality healthcare facilitate transmission of *Salmonella enterica* serovars Typhi and Paratyphi. Despite global reductions in disease burden following improvements in water, sanitation, and vaccination programmes, millions of new infections and thousands of deaths continue to occur annually, with Africa accounting for a substantial proportion of the burden. Recent global estimates indicate that enteric fever remains an important cause of morbidity and mortality, particularly among children and young adults living in endemic settings (1-5).

In Nigeria, enteric fever continues to be one of the most frequently diagnosed causes of prolonged febrile illness. The epidemiology of the disease is influenced by poor environmental sanitation, inadequate potable water supply, indiscriminate antimicrobial use, and

weak laboratory diagnostic capacity. Although clinical diagnosis is common, the nonspecific presentation of enteric fever overlaps considerably with malaria, invasive bacterial infections, and other febrile illnesses, making laboratory confirmation essential for appropriate patient management (4,6–8).

Blood culture remains the accepted reference standard for laboratory diagnosis of enteric fever because it provides viable isolates for antimicrobial susceptibility testing and epidemiological surveillance. However, the sensitivity of blood culture is limited by several factors, including low bacterial concentration in blood, prior antimicrobial exposure, delayed presentation to healthcare facilities, and inadequate laboratory infrastructure. Consequently, many diagnostic laboratories in resource-limited settings rely heavily on conventional biochemical identification following culture isolation (9-11).

Routine phenotypic identification of *Salmonella* species is based primarily on colony morphology, carbohydrate fermentation patterns, hydrogen sulfide production, motility, citrate utilization, urease activity, lysine decarboxylation, and triple sugar iron (TSI) agar reactions. Although these methods are inexpensive and widely available, several members of the family *Enterobacteriaceae*, including *Citrobacter*, *Enterobacter*, *Klebsiella*, and related genera, may exhibit similar biochemical characteristics. Such phenotypic overlap frequently results in false-positive identification of *Salmonella*, leading to inaccurate diagnosis, inappropriate antimicrobial therapy, and distorted surveillance data (12-15).

The introduction of molecular identification methods has substantially improved the accuracy of bacterial taxonomy. Among these, sequencing of the 16S ribosomal RNA (16S rRNA) gene has become one of the most widely used approaches for bacterial identification because the gene contains highly conserved regions suitable for universal amplification as well as variable regions that permit differentiation among bacterial taxa. Comparison of 16S rRNA gene sequences with curated international databases enables reliable identification of bacterial isolates that cannot be confidently resolved using conventional biochemical methods alone (16-18).

Beyond species identification, 16S rRNA sequencing also provides valuable phylogenetic information by elucidating evolutionary relationships among bacterial isolates. Although the technique lacks sufficient discriminatory power for detailed serovar differentiation compared with whole-genome sequencing, it remains an important molecular tool for confirming bacterial identity, validating phenotypic diagnoses, and supporting epidemiological investigations, particularly in laboratories where advanced genomic technologies are not readily available (17,19,20).

Accurate laboratory identification of *Salmonella* has become increasingly important in the era of antimicrobial resistance. Misidentification of non-*Salmonella* Enterobacteriaceae as *Salmonella* may result in erroneous estimates of disease prevalence, inaccurate antimicrobial resistance surveillance, and inappropriate public health interventions. Integration of molecular confirmation into routine diagnostic workflows therefore represents an important strategy for improving surveillance quality and guiding evidence-based antimicrobial stewardship programmes (3,21-24).

This study evaluated the performance of conventional phenotypic identification methods compared with 16S rRNA gene sequencing for the identification of *Salmonella* isolates recovered from patients with suspected enteric fever attending selected healthcare facilities in Nigeria. Specifically, the study determined the prevalence of culture-confirmed *Salmonella*, assessed the diagnostic performance of routine biochemical identification relative to molecular confirmation, characterized organisms responsible for phenotypic misclassification, and evaluated the phylogenetic relationships among molecularly confirmed *Salmonella* isolates. The findings provide evidence for strengthening laboratory diagnosis and improving surveillance of enteric fever in resource-limited settings.

Materials and method:

Study design and setting:

This study employed a hospital-based cross-sectional laboratory investigation to evaluate the performance of conventional phenotypic identification methods relative to 16S rRNA gene sequencing for the identification of *Salmonella* isolates recovered from blood cultures of patients with suspected enteric fever. This study was conducted in Keffi Local Government Area (LGA), Nasarawa State, Nigeria. Keffi is a peri-urban setting with a population characterized by rapid growth, limited water and sanitation infrastructure, and high burdens of communicable diseases.

The study site was selected due to its endemicity for enteric fever and the availability of secondary and tertiary healthcare facilities that manage large volumes of febrile patients. The study was carried out over a defined surveillance period of six months, coinciding

with both rainy and dry seasons to capture temporal variation in infection risk.

Study participant recruitment:

Patients presenting to selected health facilities with febrile illness were screened for eligibility. Inclusion criteria were; (i) age 1–65 years, (ii) fever ≥38 °C persisting for more than three days, and (iii) clinical features and complications suspicion of enteric fever by attending clinicians. These clinical features and complications included abdominal pain, diarrhoea or constipation, abdominal swelling, anorexia, vomiting, furring of the tongue, headache, bone pains, fatigability, pallor, meningeal irritation signs, rash, convulsion, and altered consciousness.

Exclusion criteria included prior antibiotic therapy within 72 hours, known chronic illness (e.g., HIV, TB), or refusal to consent. A total of 425 consecutive patients meeting inclusion criteria were enrolled, reflecting the typical patient load for febrile illnesses in the region. Informed consent was obtained from adult participants or guardians of minors, with ethical approval granted by the FMCK Health Research Ethics Committee.

Ethical consideration:

Ethical approval was obtained from the FMC Keffi Health Research Ethics Committee. All study procedures complied with the Declaration of Helsinki and Nigerian National Ethics guidelines. Participation was voluntary, and confidentiality was maintained.

Data collection:

A structured, interviewer-administered questionnaire captured demographic and clinical data. Data collectors were trained research assistants who conducted interviews in English or local languages. The questionnaire was pretested for reliability and adapted from validated tools used in similar epidemiological studies (26,27).

Diagnostic workflow for phenotypic and molecular identification of *Salmonella:*

A structured diagnostic workflow was applied, integrating routine phenotypic testing with molecular confirmation. The workflow illustrates the progression from blood culture and biochemical screening to molecular verification and highlights stages where phenotypic misclassification occurred (Fig 1). Fig 2 showed the diagnostic algorithm illustrating phenotypic-to-molecular confirmation of *Salmonella* identification.

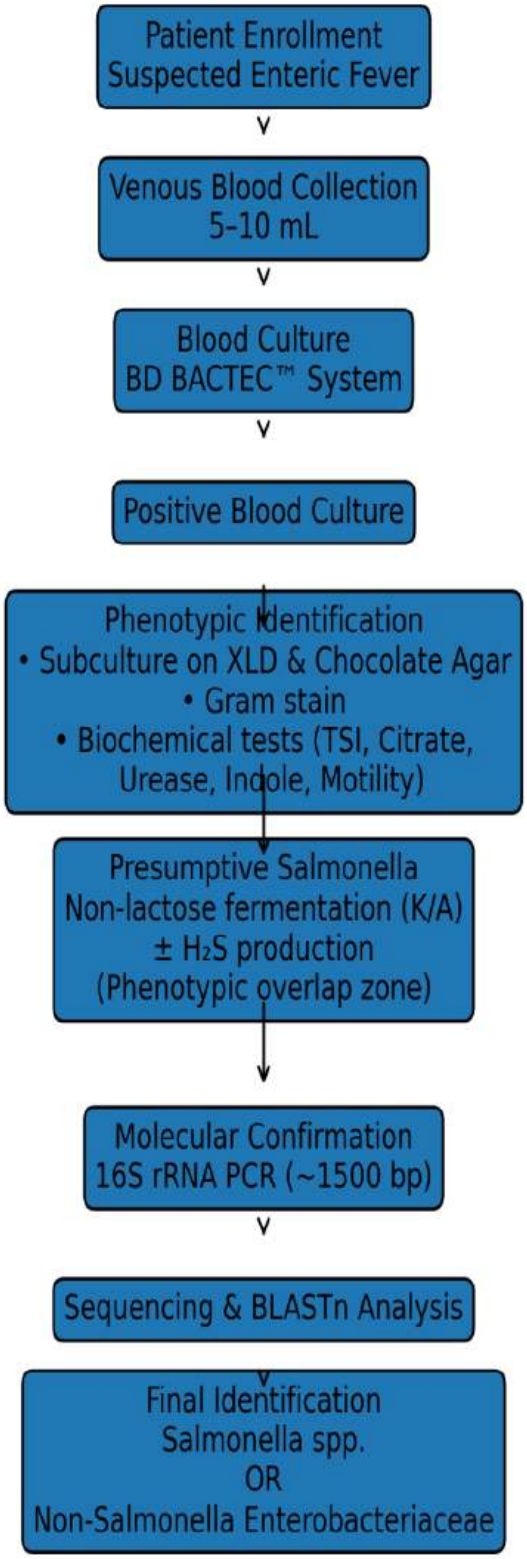

Fig 1: Workflow of phenotypic and molecular identification of S*almonella*

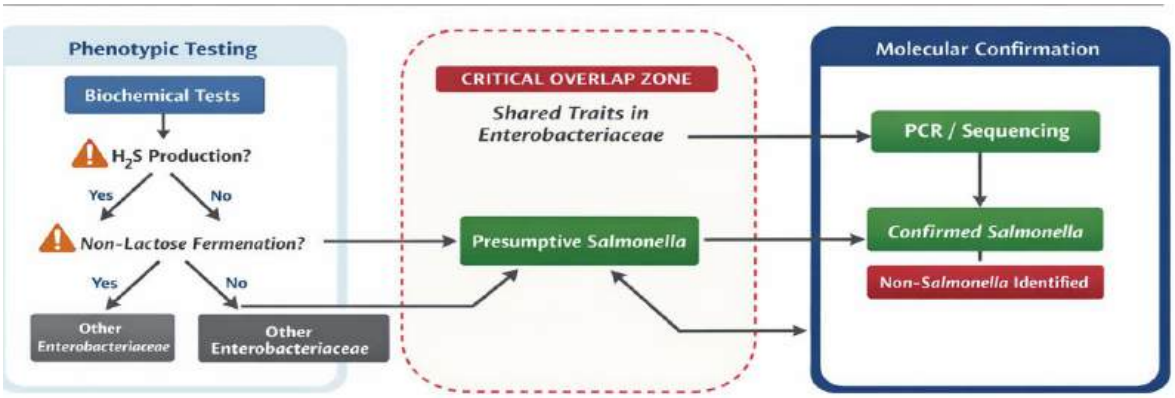

Fig 2: Diagnostic algorithm illustrating phenotypic-to-molecular confirmation of Salmonella identification

Stepwise diagnostic algorithm depicting the workflow for identification of presumptive Salmonella isolates. Initial classification was based on conventional phenotypic testing, including biochemical reactions indicative of non-lactose fermentation and hydrogen sulfide (H_2S) production. Due to overlapping phenotypic traits among members of the family Enterobacteriaceae, some non-Salmonella isolates (e. g. Enterobacter, Klebsiella, and Citrobacter spp) met these criteria and entered a critical overlap zone. Molecular confirmation by 16S rRNA gene PCR and sequencing enabled definitive species-level identification, distinguishing true Salmonella isolates from phenotypic false positives.

Blood culture and phenotypic identification:

For each participant, 5–10 mL of venous blood was collected aseptically and inoculated into BD BACTEC™ blood culture bottles. Cultures were incubated in automated BACTEC systems and monitored for growth for up to seven days. Positive bottles were sub-cultured onto chocolate blood agar and xylose lysine deoxycholate (XLD) agar.

Colonies exhibiting typical *Salmonella*-like characteristics were further evaluated by Gram staining and stan-dard biochemical tests (Triple Sugar Iron (TSI) agar reaction, MR-VP, Urease test, Citrate utilization test, Motility test, and Indole test). Phenotypic identification was performed according to the Clinical and Laboratory Standards Institute guidelines (14) by trained microbiologists.

16S rRNA gene amplification and sequencing:

All isolates presumptively identified as *Salmonella* by phenotypic methods were subjected to molecular confirmation using 16S rRNA sequencing. DNA was extracted using a boiling method followed by ethanol precipitation. The universal primers 27F (5'-AGAGTTT GATCMTGGCTCAG-3') and 1492R (5'-TACGGY TACCTTGTTACGACTT-3') were used to amplify approximately 1,500 base pairs of the 16S rRNA gene. PCR reactions were carried out in 25 µL volumes with DreamTaq Green Master Mix (Thermo Scientific), using an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C (30 seconds), annealing at 55°C (30 seconds), extension at 72°C (90 seconds), and a final elongation at 72°C for 10 minutes. Amplicons were visualized on 1.5% agarose gels stained with ethidium bromide.

The PCR products were purified (Invitrogen PureLink PCR Purification Kit, Invitrogen/ Thermo Fisher Scientific, Baltics UAB, Vilnius, Lithuania) and sequenced bidirectionally by the Sanger sequencing (Macrogen, South Korea). Raw sequences were assembled and trimmed using BioEdit v7.2, and consensus sequences were compared to reference sequences in the NCBI GenBank database using BLASTn. Sequences with ≥99% identity and coverage were considered definitive matches for species-level classification (16,17).

Phylogenetic analysis:

To contextualize the genetic relatedness of confirmed *Salmonella* isolates, representative 16S rRNA gene sequences were aligned with global reference strains retrieved from GenBank. Multiple sequence alignment was performed using ClustalW in MEGA v11, and phylogenetic trees were constructed using the Maximum Likelihood method with 1,000 bootstrap replicates (24,25). Trees were rooted with *Escherichia coli* K-12 as an outgroup to confirm evolutionary placement (21,24). Bootstrap values ≥70% were considered to indicate strong phylogenetic support (25).

Quality assurance:

Laboratory procedures adhered to standard biosafety practices. Positive and negative controls were included in each PCR batch. To minimize cross-contamination, DNA extraction, PCR setup, and post-amplification handling were conducted in separate rooms with dedicated pipettes. Sequences were checked for contami-

nation by comparing against both forward and reverse primers and excluding chimeric sequences.

Data analysis:

Data were analyzed using SPSS version 26.0. Participant characteristics were summarized using descriptive statistics, with categorical variables presented as frequencies and percentages and continuous variables as means ± standard deviations. Concordance between phenotypic identification and molecular confirmation was assessed using 2×2 contingency tables, with 16S rRNA gene sequencing as the reference standard.

Diagnostic performance of phenotypic methods was evaluated by calculating sensitivity, specificity, positive and negative predictive values with 95% confidence intervals. Agreement beyond chance between phenotypic identification and molecular confirmation was quantified using Cohen's kappa (κ) statistic. Misclassified isolates and patterns of phenotypic overlap were summarized descriptively, and phylogenetic relationships among confirmed *Salmonella* isolates were assessed using 16S rRNA gene sequence–based analysis. A *p*-value < 0.05 was considered statistically significant.

Results:

Participant characteristics:

A total of 425 patients with suspected enteric fever were enrolled. The mean age was 22.1±14.4 years (range: 1–65 years), and females accounted for 54.6% (232/425) of participants. All patients presented with febrile illness lasting more than three days. Demographic characteristics are summarized in Table 1.

Bood culture yield and phenotypic identification:

Of the blood cultures processed for 425 selected patients, 63 (14.8%) yielded bacterial growth, giving a microbiologically documented prevalence of 14.8%. Based on routine phenotypic identification using selective media and biochemical assays, 32 isolates (50.8% of culture-positive samples and 7.5% of blood cultures) were identified as *Salmonella* spp while 31 isolates were identified as non-*Salmonella* pathogens.

Molecular confirmation by 16S rRNA gene amplification and sequencing:

All 32 phenotypically identified *Salmonella* isolates subjected to 16S rRNA gene amplification and sequencing, amplified to the nearly full-length 16S rRNA gene (~1,500 bp), as shown in agarose gel electrophoresis (Fig 3). Molecular analysis confirmed 25 isolates (78.1%) as *Salmonella* spp with ≥ 99% sequence identity, while 7 isolates (21.9%) were reclassified as non-*Salmonella* Enterobacteriaceae.

Table 1: Demographic characteristics of study participants

General characteristics	Sex		Total (n=425)	χ^2	p-value
	Male (n=193)	Female (n=232)			
Age group (years)					
1-10	66 (34.2)	52 (22.4)	118 (27.8)	20.737	0.001
11-20	41 (21.2)	50 (21.6)	91 (21.4)		
21-30	33 (17.1)	79 (34.1)	112 (26.4)		
31-40	28 (14.5)	33 (14.2)	61 (14.4)		
41-50	14 (7.3)	13 (5.6)	27 (6.4)		
>50	11 (5.7)	5 (2.2)	16 (3.8)		
Mean age (± SD)	21.66±16.22	22.50±12.67	22.12±14.38	0.597	0.551

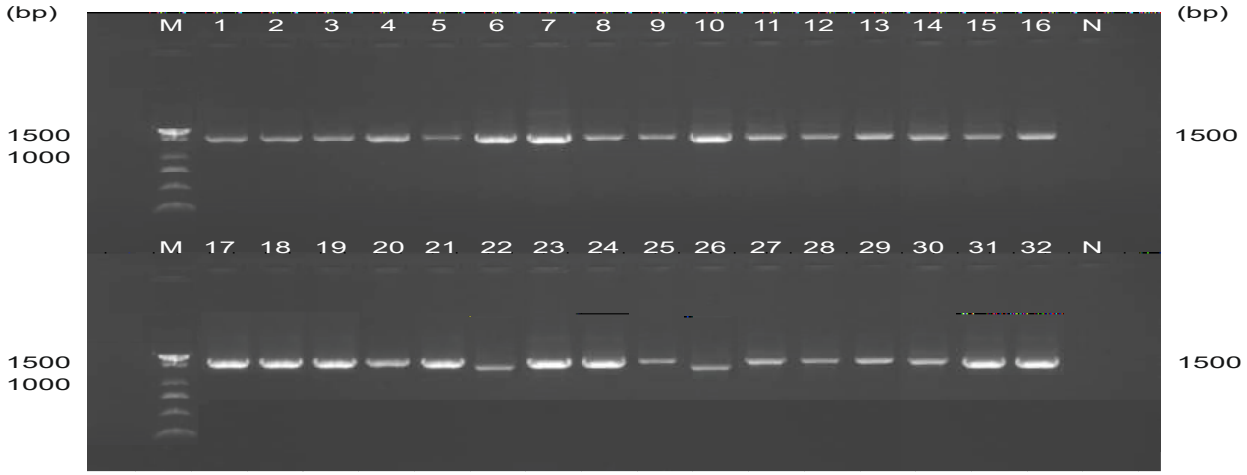

Fig 3: Agarose gel electrophoresis of 16S rRNA gene PCR products from phenotypic *Salmonella* isolates

Representative 1.5% agarose gel showing PCR amplification of the nearly full-length 16S rRNA gene (~1,500 bp) from 32 isolates initially identified as Salmonella by phenotypic methods using universal primers 27F and 1492R. Lanes 1–32 represent PCR amplicons from individual isolates. Lane M contains a 250 bp DNA ladder (Promega), and lane N represents the negative control, which showed no amplification.

Table 2: Concordance between phenotypic and molecular identification of blood culture isolates

Phenotypic identification	**Molecular confirmed *Salmonella***	**Molecular-confirmed Non-*Salmonella***	**Total**
Salmonella	25 (True positives)	7 (False positives)	32
Non-*Salmonella*	0 (False negatives)	31 (True negatives)	31
Total	25	38	63

Note: Cohen's kappa coefficient (κ) = 0.78 ($p < .001$), indicating substantial agreement between phenotypic and molecular identification methods.

Phenotypic–genotypic concordance and diagnostic performance:

Comparison of phenotypic identification with molecular confirmation via 16S rRNA gene sequencing, as summarized in Table 2, revealed substantial agreement between the two diagnostic approaches (Cohen's κ=0.78, $p<0.001$). Among 32 isolates initially classified as *Salmonella* by conventional phenotypic methods, 25 (78.1%) were confirmed as true *Salmonella*. However, 7 isolates (21.9%) were identified as false positive *Salmonella*, representing non-*Salmonella* Enterobacteriaceae that were misclassified due to overlapping biochemical traits.

Importantly, no false-negative phenotypic identifications were observed; all 31 isolates initially categorized as non-*Salmonella* by routine testing were unequivocally confirmed as true negatives by sequencing. These findings indicate that while phenotypic screening is highly reliable for excluding *Salmonella* (NPV= 100%), its imperfect specificity results in clinically meaningful overestimation of the disease prevalence, reinforcing the necessity of molecular confirmation for definitive species-level identification.

Table 3: Diagnostic performance of phenotypic identification of *Salmonella* compared to 16S rRNA sequencing

Parameter	**Estimate (%)**	**95% Confidence Interval (Clopper-Pearson)**
Sensitivity	100.0	84.6 – 100.0
Specificity	81.6	66.6 – 91.6
PPV	78.1	60.0 – 90.7
NPV	100.0	88.8 – 100.0

Reference standard: 16S rRNA gene sequencing
Sample size: n = 63 bloodstream isolates
PPV = Positive Predictive Value
NPV=Negative Predictive Value

Using 16S rRNA gene sequencing as the reference standard, the diagnostic performance of phenotypic identification for *Salmonella* was comprehensively evaluated (Table 3). The method demonstrated sensitivity of 100.0% (95% CI: 84.6–100.0), confirming that no culture-positive *Salmonella* case was missed by conventional biochemical testing, a reflection of the zero false-negative results observed in our concordance analysis. The specificity was 81.6% (95% CI: 66.6–91.6), indicating that while phenotypic tests correctly excluded majority of non-*Salmonella* isolates, they

still misclassified 7 of 38 molecular-confirmed non-*Salmonella* Enterobacteriaceae as false positives. Accordingly, the positive predictive value (PPV) was 78.1% (95% CI: 60.0–90.7) meaning that approximately one in five presumptive *Salmonella* isolates identified by routine methods would ultimately require molecular correction. In contrast, the negative predictive value (NPV) was 100.0% (95% CI: 88.8–100.0), reinforcing that a negative phenotypic result is entirely conclusive and reliably rules out *Salmonella* bacteremia.

Collectively, these recalculated metrics correcting the earlier overestimation of false positives and underestimation of specificity highlight that while phenotypic screening serves as a highly sensitive initial tool with an excellent rule-out capacity; its imperfect specificity creates a clinically meaningful risk of overdiagnosis. This underscores the critical need for molecular confirmation to guide appropriate antimicrobial therapy and accurate disease surveillance in endemic settings. The extent of phenotypic misclassification and molecular confirmation is summarized graphically in Fig 4, illustrating the proportion of true-positive and false-positive *Salmonella* identifications.

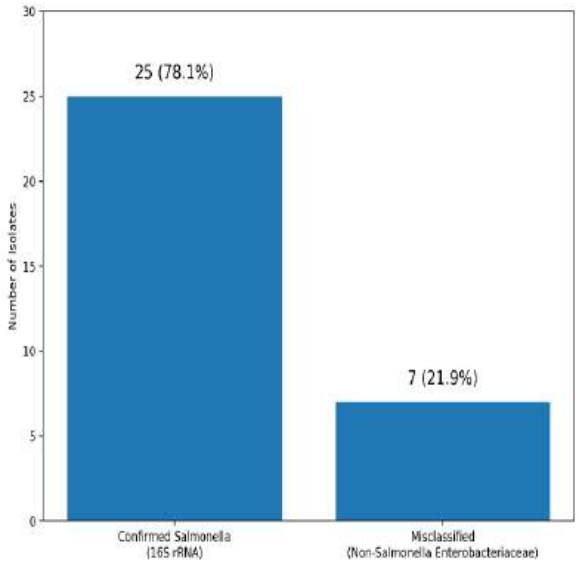

Fig 4: Phenotypic–genotypic concordance of presumptive *Salmonella* isolates

Misclassified isolates and patterns of phenotypic overlap:

The 16S rRNA gene sequence analysis demonstrated that only 25 of the 32 presumptive *Salmonella* isolates were correctly identified, corresponding to molecular prevalence of 5.9% among all clinical specimens. The remaining 7 isolates initially classified as *Salmonella* by phenotypic methods were misidentified and were found to belong to other members of the family *Enterobacteriaceae*, indicating that conventional phenotypic methods had overestimated the prevalence of *Salmonella* infection.

The confirmed *Salmonella* isolates comprised 11 isolates of *Salmonella* Typhimurium*,* 6 isolates identified as *Salmonella enterica* without further serovar assignment, and 1 isolate each of *Salmonella* Enteritidis, *Salmonella* Heidelberg, *Salmonella* Infantis, *Salmonella* Typhi*, Salmonella* Livingstone*, Salmonella* Para typhi B*, Salmonella diarizonae*, and *Salmonella bongori* (Table 4). The 7 phenotypically misidentified isolates were characterized by molecular method as 3 *Enterobacter oligotrophicus*, 1 *Enterobacter hormaechei*, 1 *Citrobacter freundii*, 1 *Citrobacter youngae*, and 1 *Klebsiella oxytoca*. These findings demonstrate the inability of phenotypic identification alone to reliably distinguish *Salmonella* from closely related members of the family Enterobacteriaceae (Table 5).

Table 6 presents a side-by-side comparison of molecular identifications and key biochemical reactions for all 32 isolates initially presumptively identified as *Salmonella* by phenotypic methods. Among these, molecular confirmation by 16S rRNA gene sequencing established that 25 isolates (78.1%) were genuine *Salmonella* species, while 7 isolates (21.9%) were reclassified as other members of the Enterobacteriaceae. Strikingly, all 25 confirmed *Salmonella* isolates comprising diverse serovars including *S.* Typhimurium, *S. enterica* (untyped), *S.* Enteritidis, *S.* Typhi*, S.* Paratyphi B, *S.* Infantis, *S.* Livingstone*, S.* Heidelberg*, S. diarizonae*, and *S. bongori* exhibited the classic biochemical profile of non-lactose fermentation (K/A reaction on TSI agar) and hydrogen sulfide (H_2S) production. Consequently, all were categorized under the "Typical *Salmonella*" phenotypic interpretation, underscoring that the conventional biochemical algorithm performed perfectly for this subset of true positives.

Table 4: Molecular identification of presumptive Salmonella isolates by 16S rRNA sequencing

Molecular identification	Number of isolates	Percentage of confirmed isolates (%)
Salmonella Typhimurium	11	44.0
Salmonella enterica (untyped)	6	24.0
Salmonella Enteritidis	1	4.0
Salmonella Heidelberg	1	4.0
Salmonella Infantis	1	4.0
Salmonella Typhi	1	4.0
Salmonella Livingstone	1	4.0
Salmonella Paratyphi B	1	4.0
Salmonella diarizonae	1	4.0
Salmonella bongori	1	4.0
Total confirmed *Salmonella*	**25**	**100.0**

Table 5: Non-*Salmonella* Enterobacteriaceae isolates misidentified as *Salmonella* by phenotypic methods and reclassified by 16S rRNA gene sequencing

Phenotypic identification	True identity (by16S rRNA sequencing)	Number
Salmonella spp	*Enterobacter Oligotrophicus*	3
Salmonella spp	*Enterobacter hormaechei*	1
Salmonella spp	*Citrobacter freundii*	1
Salmonella spp	*Citrobacter youngae*	1
Salmonella spp	*Klebsiella oxytoca*	1
Total		**7**

In contrast, the 7 misidentified non-*Salmonella* isolates demonstrated heterogeneous biochemical profiles that accounted for their diagnostic confusion. Three of these *Citrobacter freundii* (S7), *Klebsiella oxytoca* (S27), and *Enterobacter hormaechei* (S32) were particularly deceptive, as they not only fermented non-lactose (K/A) but also produced hydrogen sulfide (H_2S+), thereby creating a complete phenotypic mimicry of *Salmonella* and placing them in the "high-risk misidentification" category. The remaining 4 false positives *Citrobacter youngae* (S22) and 3 *Enterobacter oligotrophicus* isolates (S28–S30) were H_2S-negative (H_2S-) yet were still misclassified as *Salmonella*, suggesting that other overlapping biochemical traits, such as urease activity, citrate utilization, or motility patterns, may have been interpreted ambiguously or overlooked during routine screening.

Collectively, these findings demonstrate that while the combination of non-lactose fermentation and H_2S production reliably identified true *Salmonella* in this cohort, the occurrence of H_2S-producing non-*Salmonella* organisms represents a significant diagnostic pitfall, and conventional phenotypic algorithms cannot be solely relied upon for definitive species-level identification without molecular corroboration.

Phylogenetic validation of confirmed *Salmonella* isolates:

Phylogenetic reconstruction based on partial 16S rRNA gene sequences demonstrated considerable genetic diversity among the confirmed *Salmonella* isolates while maintaining clear clustering within the genus. Four principal clades were identified (Figs 5 and 6).

The first and largest clade consisted predominantly of *S.* Typhimurium isolates together with several *Salmonella enterica* strains, indicating close evolutionary relatedness among these organisms. Additional clades contained *S.* Enteritidis, *S.* Heidelberg, *S.* Infantis, *S.* Livingstone, and *S. bongori*, while *S.* Typhi and *S.* Paratyphi B clustered within a strongly supported basal lineage.

Table 6: Comparison of molecular identification and key biochemical characteristics

Sample ID	Molecular identification	Confirmed *Salmonella*	TSI reaction (Slant/Butt)	Non-lactose fermentation (K/A)	H_2S	Likely phenotypic interpretation
S1	*S.* Livingstone	Yes	K/A	Yes	+	Typical *Salmonella*
S2	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S3	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S4	*S.* Heidelberg	Yes	K/A	Yes	+	Typical *Salmonella*
S5	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S6	*S.* Enteritidis	Yes	K/A	Yes	+	Typical *Salmonella*
S7	*Citrobacter freundii*	No	K/A	Yes	+	High-risk misidentification
S8	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S9	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S10	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S11	*S.* Paratyphi B	Yes	K/A	Yes	+	Typical *Salmonella*
S12	*S.* Typhi	Yes	K/A	Yes	+	Typical *Salmonella*
S13	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S14	*S. bongori*	Yes	K/A	Yes	+	Typical *Salmonella*
S15	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S16	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S17	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S18	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S19	*S. enterica* (untyped)	Yes	K/A	Yes	+	Typical *Salmonella*
S20	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S21	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S22	*Citrobacter youngae*	No	K/A	Yes	−	False presumptive *Salmonella*
S23	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S24	*S. diarizonae*	Yes	K/A	Yes	+	Typical *Salmonella*
S25	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S26	*S.* Infantis	Yes	K/A	Yes	+	Typical *Salmonella*
S27	*Klebsiella oxytoca*	No	K/A	Yes	+	High-risk misidentification
S28	*Enterobacter oligotrophicus*	No	K/A	Yes	−	False presumptive *Salmonella*
S29	*Enterobacter oligotrophicus*	No	K/A	Yes	−	False presumptive *Salmonella*
S30	*Enterobacter oligotrophicus*	No	K/A	Yes	−	False presumptive *Salmonella*
S31	*S.* Typhimurium	Yes	K/A	Yes	+	Typical *Salmonella*
S32	*Enterobacter hormaechei*	No	K/A	Yes	+	High-risk misidentification

Confirmed *Salmonella* = Molecularly identified as *Salmonella* spp.
Non-*Salmonella* = Molecularly identified as other Enterobacteriaceae
TSI pattern: K/A = non-lactose fermenter; A/A = lactose/sucrose fermenter
H_2S: + = hydrogen sulfide production

Bootstrap values ranged from approximately 68% to 89% across the principal internal branches, while the basal lineage containing *S.* Typhi*, S.* Paratyphi B, and *S.* Typhimurium demonstrated very strong support (96–100%). These findings indicate that although the circulating isolates exhibited genetic diversity, they remained closely related evolutionarily and were clearly distinguishable from the non-*Salmonella* isolates identified by sequencing.

Overall, 16S rRNA sequencing reduced the estimated prevalence of *Salmonella* infection from 7.5% to 5.9% by eliminating seven false-positive phenotypic identifications. In addition to improving diagnostic accuracy, molecular characterization provided detailed information on species diversity and evolutionary relationships that could not be obtained using conventional laboratory methods alone.

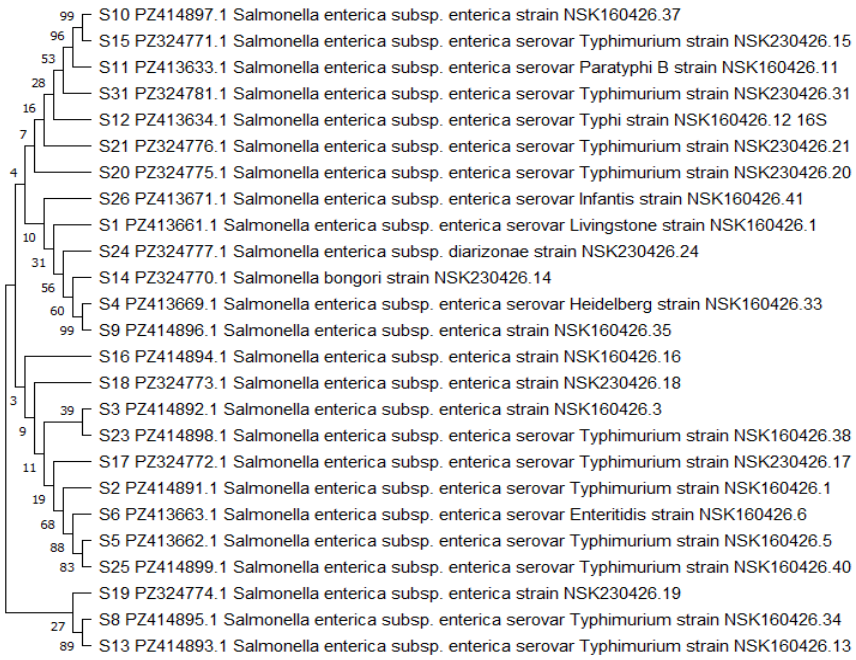

Fig 5: Phylogenetic tree of the 25 molecular confirmed *Salmonella* isolates with bootstraps

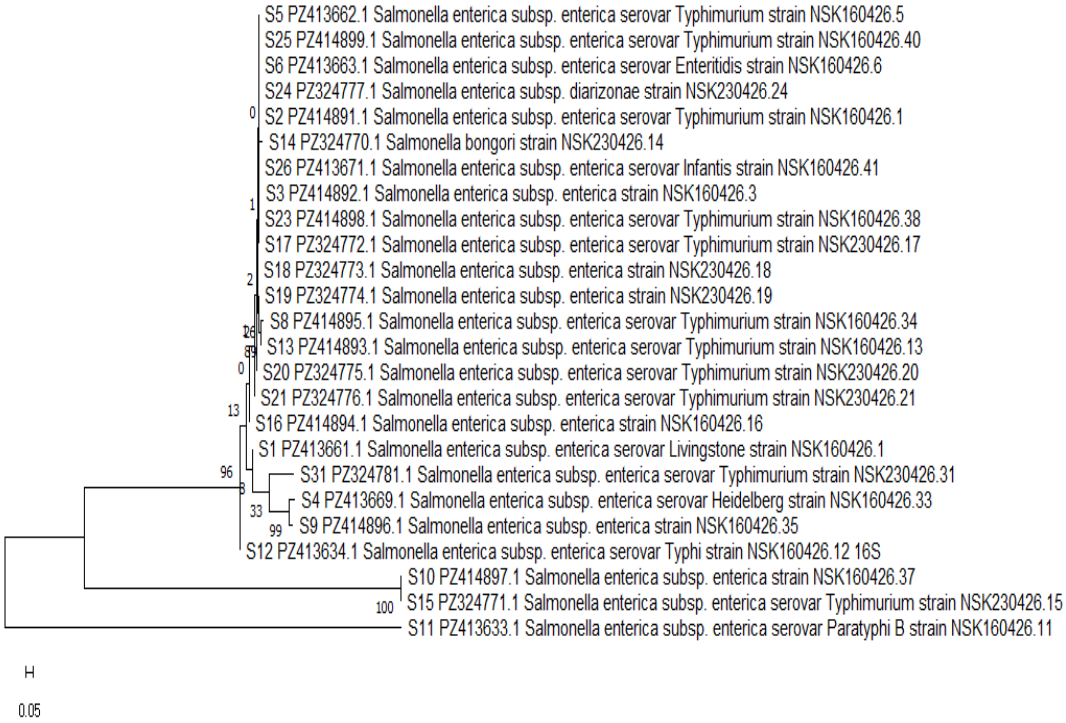

Fig 6: Phylogenetic tree of the 25 molecular confirmed *Salmonella* isolates without bootstraps

Discussion:

This study evaluated the diagnostic performance of conventional phenotypic methods relative to 16S rRNA gene sequencing for the identification of *Salmonella* isolates recovered from blood cultures of patients with suspected enteric fever in Nigeria. The findings demonstrate that although routine biochemical methods remain useful as an initial screening

approach, they substantially overestimate *Salmonella* prevalence because of phenotypic similarities shared with other members of the family Enterobacteriaceae. Molecular confirmation using 16S rRNA sequencing significantly improved species identification, revealed important patterns of phenotypic misclassification, and provided phylogenetic evidence supporting the diversity of circulating *Salmonella* strains.

The blood culture positivity observed in this study is comparable to reports from other endemic regions of sub-Saharan Africa, where culture yields are frequently limited by prior antimicrobial exposure, delayed presentation to healthcare facilities, low-level bacteremia, and inadequate laboratory infrastructure (1-5). Although blood culture remains the reference standard for laboratory diagnosis of enteric fever, its sensitivity alone does not eliminate the possibility of species misidentification when isolate characterization relies exclusively on biochemical methods. Consequently, culture-positive isolates require accurate species confirmation before they are used for disease surveillance or antimicrobial resistance reporting.

One of the most important findings of this study was the discrepancy between phenotypic identification and molecular confirmation. Conventional biochemical testing initially classified all presumptive isolates as *Salmonella*; however, 16S rRNA gene sequencing demonstrated that a considerable proportion of these isolates were in fact members of other genera, including *Enterobacter*, *Citrobacter*, and *Klebsiella*. These findings are consistent with previous reports showing that several Enterobacteriaceae possess overlapping biochemical characteristics, including non-lactose fermentation, hydrogen sulphide production, and similar triple sugar iron (TSI) agar reactions, making differentiation by routine biochemical testing challenging (12,15-19).

The occurrence of false-positive *Salmonella* identification has important clinical implications. Patients may receive unnecessary or inappropriate antimicrobial therapy when treatment decisions are based solely on presumptive biochemical identification. Such inappropriate antimicrobial use contributes to selective pressure for antimicrobial resistance, increases healthcare costs, and exposes patients to avoidable adverse drug reactions. Furthermore, inaccurate identification compromises antimicrobial resistance surveillance by attributing resistance profiles to *Salmonella* when they may originate from other clinically significant Enterobacteriaceae (3,20,28,29).

The diagnostic performance analysis further demonstrated that conventional biochemical methods possessed high sensitivity but comparatively lower specificity when evaluated against 16S rRNA sequencing. This finding indicates that while phenotypic screening is useful for minimizing missed *Salmonella* infections, it is insufficient as a definitive diagnostic method because of its susceptibility to false-positive results. The moderate agreement observed between phenotypic and molecular identification, as reflected by the Cohen's kappa statistic, reinforces the need for selective molecular confirmation, particularly for isolates intended for epidemiological surveillance, outbreak investigations, and antimicrobial resistance monitoring. Similar conclusions have been reported by recent studies evaluating molecular confirmation of foodborne pathogens in both clinical and public health laboratories (16-19,30).

Molecular sequencing identified several non-*Salmonella* organisms that had been incorrectly classified by biochemical methods. Species of *Enterobacter*, *Citrobacter*, and *Klebsiella* are well-recognized opportunistic pathogens capable of causing bloodstream infections, urinary tract infections, neonatal sepsis, and healthcare-associated infections. Their phenotypic resemblance to *Salmonella* has been documented previously and remains an important challenge in laboratories lacking access to molecular diagnostic technologies (15-19). Correct identification of these organisms is essential because they differ substantially from *Salmonella* in their epidemiology, virulence characteristics, and antimicrobial susceptibility patterns.

Phylogenetic analysis provided independent confirmation of the molecular identifications. The 16S rRNA gene sequences of confirmed isolates clustered closely with reference *Salmonella enterica* sequences, supporting the reliability of species assignment. The phylogenetic tree further demonstrated genetic diversity among circulating isolates through the presence of multiple well-supported clades, suggesting the circulation of several *Salmonella* lineages within the study population. Such genetic diversity has been increasingly reported in African surveillance studies and reflects the complex epidemiology of invasive salmonellosis in the region (3,9,14,31,32). Although 16S rRNA sequencing does not possess sufficient discriminatory power to differentiate closely related serovars or detect antimicrobial resistance genes, it remains a robust and cost-effective approach for confirming bacterial identity and validating routine microbiological diagnosis where whole-genome sequencing is not readily available (21–23). Furthermore,

even though antimicrobial susceptibility testing was not performed in this study, routine surveillance programmes should integrate standardized interpretive criteria, such as those provided by CLSI (14) and EUCAST (33), to ensure harmonized resistance monitoring across different settings.

From a public health perspective, the findings have important implications for surveillance systems in Nigeria and other resource-limited settings. Overestimation of *Salmonella* prevalence resulting from phenotypic misclassification may distort national disease burden estimates, bias antimicrobial resistance surveillance, and influence treatment guidelines and public health policy. Integrating molecular confirmation into routine laboratory workflows particularly within sentinel surveillance laboratories would substantially improve the quality of epidemiological data while supporting national antimicrobial resistance control programmes and the World Health Organization Global Antimicrobial Resistance and Use Surveillance System (GLASS) (20,29), as well as established programmes like the US CDC's National Antimicrobial Resistance Monitoring System (NARMS) (34).

The major strength of this study lies in the application of molecular sequencing to validate routine biochemical identification and characterize the phylogenetic relationships among clinical isolates. Unlike studies relying solely on conventional microbiology, the present investigation was able to identify false-positive *Salmonella* isolates and demonstrate the diversity of circulating strains. Nevertheless, several limitations should be acknowledged. The study was conducted within a limited geographical area, and the number of molecularly analysed isolates may not fully represent the diversity of *Salmonella* circulating nationally. In addition, identification based on 16S rRNA sequencing cannot reliably distinguish all *Salmonella* serovars or characterize antimicrobial resistance determinants.

While 16S rRNA gene sequencing offers superior diagnostic accuracy, its routine implementation in low-resource settings faces practical challenges, including high per-sample costs, infrastructure requirements, and technical expertise. However, a tiered diagnostic strategy where conventional biochemical methods serve as an initial screening tool, and molecular confirmation is reserved for surveillance activities, outbreak investigations, and isolates intended for antimicrobial resistance monitoring presents a pragmatic and cost-effective approach. This selective application minimizes costs while maximizing public health impact.

Compared to commercial automated identification systems such as API, Microbact, or VITEK, which are also cost-prohibitive for many laboratories in Nigeria, 16S rRNA sequencing offers the added advantage of providing phylogenetic information and enabling the detection of novel or emerging pathogens. As sequencing costs continue to decline and capacity-building initiatives expand across sub-Saharan Africa, molecular confirmation is becoming increasingly feasible for sentinel surveillance laboratories. Future studies employing whole-genome sequencing, multi-locus sequence typing, or core-genome phylogenetic analysis would provide higher-resolution epidemiological data to further strengthen these surveillance efforts.

Overall, the findings demonstrate that conventional biochemical methods remain valuable for initial laboratory screening but should not be relied upon as the sole basis for definitive identification of *Salmonella*. A tiered diagnostic strategy incorporating routine phenotypic screening followed by selective molecular confirmation would provide a practical and cost-effective approach for improving diagnostic accuracy, strengthening antimicrobial resistance surveillance, and enhancing enteric fever control programmes in endemic settings.

Conclusion:

This study demonstrates that conventional phenotypic methods, although useful for the initial screening of *Salmonella* isolates, are insufficient for definitive species identification because of substantial biochemical overlap with other members of the family Enterobacteriaceae. Molecular confirmation using 16S rRNA gene sequencing significantly improved diagnostic accuracy by distinguishing true *Salmonella* isolates from phenotypically similar organisms, thereby reducing the risk of misclassification.

The study further showed that reliance on biochemical identification alone can lead to overestimation of *Salmonella* prevalence, inaccurate antimicrobial resistance surveillance, and inappropriate clinical management. Phylogenetic analysis confirmed the taxonomic identity of the molecularly confirmed isolates and demonstrated genetic diversity among the circulating *Salmonella* strains, highlighting the value of molecular methods for epidemiological investigations.

The findings underscore the importance of incorporating molecular confirmation into routine laboratory workflows, particularly for surveillance activities, outbreak investigations,

and antimicrobial resistance monitoring. In resource-limited settings such as Nigeria, a tiered diagnostic approach combining conventional microbiological screening with selective 16S rRNA gene sequencing represents a practical and cost-effective strategy for improving diagnostic reliability and generating high-quality surveillance data.

Future studies should incorporate whole-genome sequencing and expanded multicentre surveillance to provide higher-resolution characterization of *Salmonella* serovars, transmission dynamics, virulence factors, and antimicrobial resistance determinants. Such approaches will strengthen evidence-based public health interventions and contribute to improved control of enteric fever in endemic regions.

Contributions of authors:

CJA conceptualized the research, designed the methodology, performed the experiments, and wrote the original draft; CJA, DZE and PML analysed the data, curated the figures, and reviewed the manuscript; CJA provided resources, and DZE and PML supervised the project. All three authors read and approved the published version of the manuscript.

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Authors declare no conflict of interest

Artificial Intelligence (AI) use disclosure:

During the preparation of this work the authors used AI for editing purposes. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication**.**

Data availability:

The molecular data in this study have been deposited in the NCBI GenBank database with the following accession numbers; PZ413661, PZ414891, PZ414892, PZ413669, PZ413662, PZ413663, PZ413664, PZ414895, PZ414896, PZ414897, PZ413633, PZ413634, PZ414893, PZ324770, PZ324771, PZ414894, PZ324772, PZ324773, PZ324774, PZ324775, PZ324776, PZ413665, PZ414898, PZ324777, PZ414899, PZ413671, PZ413667, PZ324778, PZ324779, PZ324780, PZ324781, and PZ413668.

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Original Article **Open Access**

Evaluation of self-collected oral swab sample for early detection of *Mycobacterium tuberculosis* by Xpert MTB assay in Karu, Nasarawa State, Nigeria

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Abstract:

Background: Tuberculosis (TB) is one of the leading causes of death worldwide, with an estimated 1.25 million people dying from TB in 2023. Most current diagnostic tests for TB rely on sputum sampling from symptomatic individuals. Sputum collection procedure involves expectoration of potentially infectious aerosols, which pose safety risks to health care workers; hence, an easier, safer, non-invasive, non-aerosol, and more effective alternative approach is warranted. This study evaluated the effectiveness and diagnostic yield of self-collected oral swab (SC-OS) against sputum in detecting *Mycobacterium tuberculosis* (MTB) by Xpert MTB/RIF assay.
Methodology: A cross-sectional comparative design was used in evaluating SC-OS against sputum for early diagnosis of TB in adolescents and adults with presumed TB in Karu Local Government Area, Nasarawa State, Nigeria. Two samples, comprising early morning SC-OS, and one sputum sample were collected from each consented participant. SC-OS and sputum were transported immediately to the laboratory at room temperature and cold chain, respectively. The Xpert MTB/RIF Ultra assay was performed on both specimens, and the graph of the cycle threshold values was plotted for both samples. The sensitivity, specificity, and predictive values of SC-OS were calculated, with sputum as the 'gold standard' sample.
Results: Of the 419 participants, 266 (63.5%) were males, and 12 (2.8%) were ≤ 14 years. Thirty-six (8.6%) were positive, with 2 (5.6%) rifampicin resistant. When compared to sputum Xpert MTB as the 'gold standard', the sensitivity and specificity of the SC-OS Xpert MTB were 97.2% and 99.7%, respectively.
Conclusion: The self-collected oral swab showed high sensitivity and specificity with the sputum sample, and is potentially easier, safer, non-invasive, non-aerosol, and effective in detecting TB by Xpert/MTB. Although further research is warranted, our findings show that SC-OS can serve as an alternative sample for early detection of *M. tuberculosis.*

Keywords: Self-collected oral swab, Sputum, Xpert MTB, Diagnostic performance

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Évaluation d'un prélèvement buccal auto-effectué pour le dépistage précoce de *Mycobacterium tuberculosis* par le test Xpert MTB à Karu, État de Nasarawa, Nigéria

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Résumé:

Contexte: La tuberculose (TB) est l'une des principales causes de mortalité dans le monde, avec environ 1,25 million de décès estimés en 2023. La plupart des tests diagnostiques actuels de la TB reposent sur le prélèvement d'expectorations chez les personnes symptomatiques. La procédure de prélèvement d'expectorations implique l'expectoration d'aérosols potentiellement infectieux, ce qui présente des risques pour la sécurité des professionnels de santé; par conséquent, une approche alternative plus simple, plus sûre, non invasive, sans aérosol et plus efficace est nécessaire. Cette étude a évalué l'efficacité et la performance diagnostique de l'écouvillon buccal auto-prélevé (EBAP) par rapport à l'expectoration pour la détection de *Mycobacterium tuberculosis* (MTB) par le test Xpert MTB/RIF.
Méthodologie: Une étude comparative transversale a été menée pour évaluer l'EBAP par rapport à l'expectoration pour le diagnostic précoce de la tuberculose chez les adolescents et les adultes présentant une suspicion de tuberculose dans la zone de gouvernement local de Karu, État de Nasarawa, au Nigéria. Deux échantillons, un EBAP prélevé tôt le matin et un échantillon d'expectoration, ont été recueillis auprès de chaque participant ayant donné son consentement. L'EBAP et l'expectoration ont été transportés immédiatement au laboratoire, respectivement à température ambiante et sous chaîne du froid. Le test Xpert MTB/RIF Ultra a été réalisé sur les deux types d'échantillons, et la courbe des valeurs de seuil de cycle (Ct) a été établie. La sensibilité, la spécificité et les valeurs prédictives de l'EBAP ont été calculées, l'expectoration servant de référence.
Résultats: Sur les 419 participants, 266 (63,5%) étaient des hommes et 12 (2,8%) avaient 14 ans ou moins. Trente-six (8,6%) étaient positifs, dont 2 (5,6%) résistants à la rifampicine. Par rapport au test Xpert MTB sur expectorations, considéré comme la méthode de référence, la sensibilité et la spécificité du test SC-OS Xpert MTB étaient respectivement de 97,2% et 99,7%.
Conclusion: L'écouvillon buccal auto-prélevé a démontré une sensibilité et une spécificité élevées par rapport à l'échantillon d'expectorations. Il est potentiellement plus simple, plus sûr, non invasif, sans aérosolisation et efficace pour la détection de la tuberculose par Xpert/MTB. Bien que des recherches supplémentaires soient nécessaires, nos résultats montrent que le SC-OS peut servir de prélèvement alternatif pour le dépistage précoce de *M. tuberculosis*.

Mots-clés: Écouvillon buccal auto-prélevé, Expectorations, Xpert MTB, Performance diagnostique

Introduction:

Tuberculosis (TB) continues to be one of the most pervasive and deadly infectious diseases worldwide, representing a formidable public health burden, particularly in low-and-middle-income countries (LMICs) (1,2). Caused by the *Mycobacterium tuberculosis* complex (MTBC), TB claimed an estimated 1.25 million lives in 2023, according to the World Health Organization (WHO) report (3). Despite significant advances in diagnostics and therapeutic interventions, TB remains entrenched as one of the top ten causes of mortality globally (2,3). The disease primarily affects the lungs, although it can disseminate to other organs, leading to extrapulmonary manifestations that are often harder to detect and manage (4)

In 2020, 86% of new TB cases were reported in 30 high-burden countries, highlighting the uneven distribution of disease burden and the urgent need for targeted interventions. Among these nations, Nigeria stands out, ranking first in Africa and sixth globally in TB burden (2,5). It is also one of the 10 countries contributing to over 70% of the global gap in TB case detection and notification (6). These statistics underscore the substantial challenges Nigeria faces in effectively diagnosing and treating TB, despite being a focus of global and national TB control efforts.

Early detection and prompt initiation of treatment are pivotal in reducing TB transmission and improving patient outcomes. However, achieving these goals is often hindered by several limitations associated with the conventional diagnostic methods (7). Currently, most TB diagnostic algorithms are centered on the detection of *M. tuberculosis* in sputum samples through microscopy, culture, or molecular techniques such as the GeneXpert MTB/RIF Ultra assay (8). While these methods have transformed TB detection, especially with the advent of molecular assays, they still rely on sputum samples, introducing several practical and biological challenges (8).

Sputum-based diagnostics pose significant limitations, particularly in populations that are either unable or unwilling to produce sputum. These include young children, elderly individuals, and people living with HIV/AIDS, who often present with paucibacillary disease (9,10). The quality of the sputum sample is another limiting factor, as poorly collected specimens can result in false negatives. (11,12). Furthermore, sputum induction procedures are aerosol-generating, raising the risk of transmission in clinical and laboratory settings, and

necessitating stringent infection control measures (13,14). These issues are further compounded in community-based screening programs, where the logistical difficulties of sample collection, storage, and transportation can delay diagnosis and treatment initiation.

Given these challenges, there has been a growing interest in alternative specimen types for TB diagnosis. Among these, oral swabs have emerged as a promising non-invasive option (15,16,17). Oral swab samples can be collected easily, without the need for specialized training or equipment, making them particularly advantageous in resource-constrained and community settings (17). Early morning self-collected oral swabs (SC-OS) are especially appealing due to their potential to empower patients, increase participation in screening programs, and facilitate decentralized TB diagnostic efforts (18,19). The concept of using oral swabs for TB diagnosis builds upon the principle that *M. tuberculosis* DNA may be expelled from the lungs and deposited on the buccal or oropharyngeal surfaces. (20) Molecular diagnostic techniques, such as the GeneXpert MTB/RIF Ultra assay, are sensitive enough to detect this DNA, even when present in low quantities (21).

Several studies (18,19,20,) have demonstrated that oral swab samples could detect MTB DNA with high specificity using molecular tools. Similarly, studies (19,20) showed that oral swabs offered a viable alternative to sputum samples, especially in patients unable to produce sputum. Another study (22) further reported that the diagnostic performance of oral swabs, when analyzed using sensitive molecular assays, was comparable to that of conventional sputum-based tests. These findings are particularly relevant for TB screening initiatives targeting populations that are difficult to reach or engage using traditional diagnostic methods. The non-invasive nature of oral swabs reduces stigma, increases compliance, and opens opportunities for large-scale community-based TB screening campaigns (23).

However, despite these promising findings, oral swab diagnostics for TB are still in the early stages of validation and implementation (16). Several operational and technical challenges need to be addressed before widespread adoption can occur. One major concern is the variability in sample quality when collected by patients themselves. While self-collection is logistically advantageous to allow for early morning (before brushing or eating) sampling, it raises concerns about the consistency and adequacy of the specimens. The use of standardized collection kits and comprehensive patient education materials is essential to mitigate this risk (24).

Another consideration is the optimization of pre-analytical and analytical protocols, including sample storage, transport, and processing. Unlike sputum, which may contain high concentrations of MTB bacilli, oral swabs may yield lower DNA quantities, necessitating highly sensitive detection methods (25). This makes the GeneXpert MTB/RIF Ultra assay an attractive option, as it offers enhanced sensitivity compared to earlier versions of the Xpert system. Yet, studies assessing the real-world performance of early morning SC-OS samples processed with GeneXpert MTB/RIF Ultra, especially in diverse populations and settings, remain limited (26).

Social stigma surrounding TB in Nigeria further complicates case detection efforts (27). Many individuals delay seeking care due to fear of discrimination or ostracization. The ability to privately self-collect oral swabs can help circumvent this barrier and encourage greater community participation in TB screening programs (28). Moreover, self-collected oral swabs are lightweight, compact, and pose a lower biohazard risk than sputum, simplifying sample transport and reducing costs associated with cold chain requirements and infection control (29). From a cost-effectiveness perspective, integrating oral swab diagnostics into existing TB programs could provide significant benefits. While the upfront cost of molecular testing platforms like GeneXpert is relatively high, the potential reduction in diagnostic delays and associated transmission can result in long-term savings (30). Furthermore, the adoption of self-collection methods could reduce reliance on trained personnel, thereby alleviating some of the human resource constraints faced by health systems in LMICs.

Importantly, while various studies have shown the potential utility of oral swabs in detecting MTB DNA, most have been conducted in clinical or laboratory-based settings. There is a paucity of data evaluating the performance of SC-OS in real-world, community-based environments, particularly in semi-urban and rural contexts where TB diagnostic gaps are most pronounced (31). As such, further research is urgently needed to assess the feasibility, diagnostic accuracy, and operational implications of this approach under field conditions. By assessing the diagnostic performance, feasibility, and operational practicality of SC-OS in this context, this research contributes valuable evidence to support the potential integration of oral swab-based testing into Nigeria's national TB control strategy. The outcomes may also

inform the development of standard operating procedures for SC-OS sample collection, transport, and processing. Ultimately, the findings from this research may have broader implications beyond Nigeria. They could inform TB diagnostic strategies in other high-burden countries facing similar challenges related to sputum-based testing and limited healthcare infrastructure. The scalability of SC-OS, coupled with the sensitivity of the GeneXpert MTB/RIF Ultra platform, presents a promising avenue for improving TB case detection, especially in marginalized and underserved populations (30).

This approach aligns with global efforts to enhance early diagnosis, decentralize care, and eliminate TB as a public health threat (32). Through rigorous scientific evaluation in a real-world setting, this study seeks to provide the evidence base needed to support the broader adoption of self-collected oral swab testing in TB-endemic regions. This study aims to address these critical knowledge gaps by evaluating the use of early morning self-collected oral swabs for the detection of *Mycobacterium tuberculosis* using the GeneXpert MTB/RIF Ultra assay in a community setting in Karu, Nasarawa State, Nigeria. Karu is a semi-urban locality comprising diverse populations with varying levels of access to healthcare services.

Materials and method:

Study design and setting:

This study utilizes an observational cross-sectional approach in evaluating early morning self-collected oral swabs for early diagnosis of TB in adolescents and adults with presumed TB in hard-to-reach communities in Karu. Routine TB testing (sputum Xpert MTB/RIF Ultra) was used for comparison with the novel tests (SC-OS). Participants were enrolled from August 2024 to February 2025 in different hard-to-reach villages in Karu Local Government Area (LGA) in Nasarawa State, Nigeria.

Karu Local Government Area sits along the busy border corridor between Nasarawa State and the Federal Capital Territory, yet beyond its urban edges lies a landscape that quickly turns rural and uneven. The area is home to a mix of ethnic groups, small farming communities, and expanding peri-urban settlements (33). Villages are surrounded by rolling hills, scattered farmland, and pockets of dense vegetation. Most households depend on small-scale farming, petty trade, and daily-wage labor to sustain their livelihoods. Markets are lively but seasonal, roads become difficult during the rains, and many communities rely on boreholes, streams, and long walks to access basic services.

Most remote communities are accessible only by motorcycle or long footpaths, and transport is often unreliable, especially during the wet season when roads become impassable. Health facilities are few, and the nearest primary health centre may require hours of travel, leaving many residents dependent on traditional healers, local chemists, and community beliefs. These hard-to-reach areas face gaps in routine healthcare, diagnostic services, and health information (34). The combination of geographic isolation, limited infrastructure, and socioeconomic constraints not only affects disease detection and treatment but also makes community engagement essential for any public health study conducted in this setting.

Study population and sample size:

The study population comprised individuals of all genders, adolescents, and adults (aged 15 years) in hard-to-reach areas of Karu LGA with either cough ≥2 weeks' duration, night sweat, weight loss, fever, and other commonly accepted criteria for identifying people with presumed pulmonary TB risk factors for which TB screening is always recommended. A total of 419 consented participants were enrolled from August 2024 to February 2025 in different communities in hard-to-reach areas of Karu LGA, Nasarawa State, Nigeria. Participants were enrolled during community outreach activities in the areas within the enrollment period.

Participant eligibility criteria:

We included adolescents and adults (aged 15 years) with either cough ≥2 weeks' duration, night sweat, weight loss, fever, and other commonly accepted criteria for identifying people with presumed pulmonary TB risk factors for which TB screening is always recommended. We excluded people who were hospitalized, and or are unwilling to provide informed consent

Data and sample collection:

Demographic and clinical data were obtained using a structured data collection form. Two samples were collected from each participant; (i) one sputum sample on the spot during outreach, and (ii) one early morning self-collected oral swab (collected the following day by the participants). Samples were transported to Zankli Research Center TB Reference Laboratory for testing. Sputum samples were transported in a cold-chain system, while the SC-OS were transported at room temperature.

Method of sputum sample collection:

Participants were asked to refrain from eating, drinking, brushing their teeth, and/or using mouthwash for at least 30 minutes before sputum sample collection. Also, the participants were asked to make a deep cough and produce good-quality sputum in a sputum cup. The demographic and clinical data of participants were collected before the sample was collected

Method of swab collection:

Participants were educated on how to collect the oral swab, which was collected early in the morning before brushing the mouth, eating, or drinking. Participants stuck out their tongue and, using a sterile, individually wrapped swab, ran the swab over the length and breadth of the front 2/3 of their tongue, and between the teeth and the tongue. Pressure was sufficient to slightly bend the swab shafts.

Samples were collected for 15-20 secs while rotating the swab throughout. The swabs were transferred into an empty disposable 15 ml Falcon tube. Each sample container was appropriately labeled with the participants information details. Informed consent/ascent was obtained from each participant before sample collection

Sputum for MTB detection by Xpert MTB/RIF:

The Xpert MTB/RIF Ultra test was performed by trained laboratory technicians at the Zankli TB Reference laboratory according to the manufacturer's instructions. A 2:1 sample reagent was added to the sputum and incubated for 15 minutes at room temperature after shaking for 30 seconds. About 2 ml was transferred into the Xpert cartridge using a sterile transfer pipette and loaded onto the Xpert machine. Results were documented at the end of the test.

Oral swab for MTB detection by Xpert MTB/RIF:

Trained personnel performed the SC-OS TB testing using the Xpert MTB/RIF Ultra according to the manufacturer's instructions and the formulated swab sample preparation. About 3 ml of the sample reagent was added to each 15 ml Falcon tube containing the SC-OS. The content was then vortexed for 20 secs and incubated for 15 minutes at room temperature. The entire liquid content was transferred into the Xpert cartridge using a sterile transfer pipette and was loaded onto the GeneXpert platform according to the manufacturer's instructions. Results were documented at the end of the test.

Ethical consideration:

Ethical approval (NHREC/21/05/2005/01309) was obtained from Bingham University Karu Ethical Committee before the commencement of this study. Eligible participants were informed of the study objectives, procedures, potential benefits, and risks in a language they understood. Written informed consent was obtained from all participants before enrolment. For participants under 18 years of age, assent was obtained along with parental or guardian consent. Each participant was assigned a unique identification code to ensure anonymity and no personal identifiers (names, addresses, or phone numbers) appeared in any database or publication.

Participants were made aware of their rights to withdraw from the study without stating any reason. Potential risks were minimal and related mainly to the collection of sputum samples, which is standard for TB diagnosis and may cause mild discomfort. The potential benefits included improved understanding of the self-collected oral swab for TB diagnosis, which could inform national TB diagnostic guidelines, improve laboratory efficiency, and enhance patient outcomes. All sputum samples were collected as part of routine TB diagnostic services, with no additional invasive procedures performed for research purposes. Results were disseminated in aggregate form only, with no possibility of linking findings to individual participants or facilities.

Data analysis:

Data were initially captured using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and subjected to double-entry verification to minimize transcription errors. Cleaning procedures included screening for missing or inconsistent values, verifying dates of sample collection and receipt, and ensuring correct coding of categorical variables. The dataset was then exported to IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) for statistical analysis.

Descriptive statistics were used to summarize demographic, clinical, and laboratory characteristics. Categorical variables (e.g., sex, age, HIV status) were presented as frequencies and percentages. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio (LR) of the SC-OS were calculated against the sputum sample Xpert MTB results as the 'gold standard'. The graph of the cycle threshold (Ct) values against positive outcomes was plotted for both sputum and SC-OS.

Results:

This study enrolled 419 participants with presumptive pulmonary TB, of whom 266 (63.5%) were male, and 12 (2.8%) were less than or equal to 14 years. Of the 419 participants, 36 (8.6%) were confirmed TB positive, with 2 (5.7%) rifampicin-resistant and 32 (91.4%) rifampicin-sensitive on both sputum and SC-OS Xpert MTB/RIF ultra, while 1 (2.9%) was rifampicin indeterminate on Xpert MTB/RIF Ultra.

For the semi-quantitative readout of the 36 positive samples, 21 (58.3%) were medium, 6 (16.7%) were low, 5 (13.9%) very-low, and 2 (5.6%) were trace on both sputum and SC-OS Xpert MTB/RIF ultra (Table 1). The sensitivity and specificity of the SC-OS compared to sputum for *M. tuberculosis* detection by Xpert MTB/RIF Ultra were 97.2% and 99.7%, respectively, while the PPV, NPV and LR were 97.2%, 99.7% and 372.4 respectively (Table 2).

Fig 1 shows the comparison of cycle threshold (Ct) values of sputum sample and that of self-collected oral swab of the positive samples on Xpert MTB/RIF assay, which showed a very high yield of *M. tuberculosis* by the SC-OS when compared to the sputum.

Table 1: Distribution of sputum Xpert MTB/RIF ultra results across gender and age of the participants and comparison with self-collected oral swab

Variable	**Sputum Xpert MTB/RIF Ultra result, RIF status, and semi-quantitative read-out**									
	Negative (%)	**Positive (%)**								
		Total	**RIF R**	**RIF S**	**RIF IND**	**High**	**Medium**	**Low**	**Very low**	**Trace**
Gender										
Male (n=266)	241 (90.6)	25 (9.3)	2 (8.0)	22 (88.0)	1 (4.0)	2 (8.0)	14 (56.0)	3 (12.0)	5 (20.0)	1 (4.0)
Female (n=153)	142 (92.8)	11 (7.1)	0	11 (100.0)	0	0	7 (63.6)	3 (27.3)	0	1 (9.1)
Age group (years)										
<14 (n=12)	6 (50.0)	6 (50.0)	1 (16.7)	5 (83.3)	0	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)
>15 (n=407)	377 (92.6)	30 (7.3)	1 (3.3)	28 (93.3)	1 (3.3)	1 (3.3)	19 (63.3)	5 (16.7)	4 (13.3)	1 (3.3)
Oral swab Xpert MTB/RIF Ultra result, RIF status, and semi-quantitative read-out										
Positive (n=36)	1 (2.7)	35 (97.2)	2 (5.7)	32 (91.4)	1 (2.9)	1 (2.9)	21 (60.0)	6 (17.1)	5 (14.3)	2 (5.7)
Negative (n=383)	382 (99.7)	1 (0.02)	0	1 (100.0)	0	1 (100.0)	0	0	0	0
Total (n=419)	**383 (91.4)**	**36 (8.6)**	**2 (5.6)**	**33 (91.7)**	**1 (2.8)**	**2 (5.6)**	**21 (58.3)**	**6 (16.7)**	**5 (13.9)**	**2 (5.6)**

RIF R = Rifampicin resistant; RIF S = Rifampicin sensitive; RIF IND = Rifampicin indeterminate

Table 2: Diagnostic performance of self-collected oral swab compared to sputum in detecting *Mycobacterium tuberculosis* by Xpert MTB/RIF ultra

Diagnostic parameter	**Xpert MTB/RIF result**	
	Self-collected oral swab	**Sputum (gold standard)**
True Positive	35	36
False Positive	1	0
True Negative	382	383
False Negative	1	0
Sensitivity (%)	97.2 (95% CI: 85.5 - 99.9)	100
Specificity (%)	99.7 (95% CI: 98.6 – 99.9)	100
Positive Predictive Value (%)	97.2 (95% CI: 85.5 – 99.9)	100
Negative Predictive Value (%)	99.7 (95% CI: 98.6 – 99.9)	100
Likelihood Ratio	372.4	

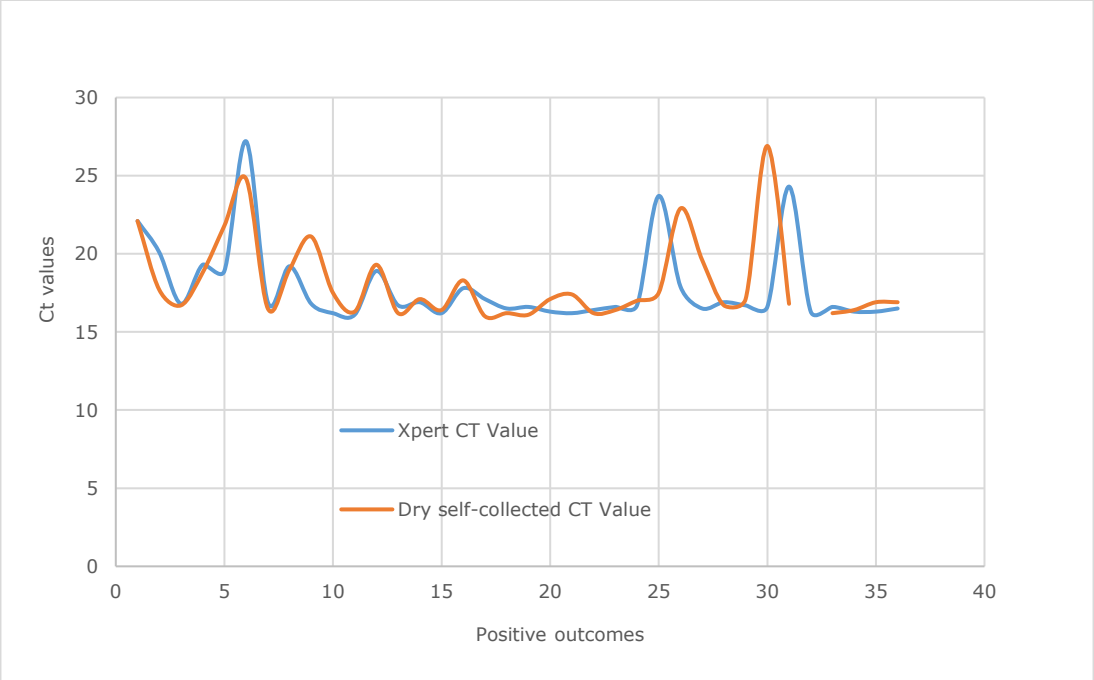

Fig 1: Comparison of the Cycle threshold (Ct) values of sputum sample and self-collected oral swab Xpert MTB assay

Discussion:

This study was performed among 419 participants in Karu, Nigeria, and focused on determining the effectiveness of early diagnosis of TB by Xpert MTB/RIF with Self-collected Oral Swabs. The results provide important data on how self-collection strategies may improve TB diagnosis, particularly in low-resource environments and hard-to-reach communities. Consistent with existing literature, which supports that men have higher TB prevalence, due to transient occupational and social behaviors (35), males made up most of the participants in this study (63.5%), and only 2.8% were aged ≤14 years. The cohort had an average of 8.6% prevalence for TB, with MTB positives in 36 participants. This result is similar to a study (36) that reported a prevalence of 5.8% per 100 participants.

The sensitivity and specificity of the SC-OS compared with sputum samples were 97.2% and 99.7%, respectively. A study by Church et al., (15) reported a sensitivity of 81.5% for oral swab in adults and 26% in children. These sound metrics suggest the method SC-OS is a robust approach to detect TB, capturing correctly most true positive cases without generating false positives. A study by Zhang et al., (16) reported a sensitivity and specificity of oral swabs at 73% and 93%, respectively. Another study by LaCourse et al., (37) reported an overall buccal swab sensitivity was 65.0% and a specificity of 81.3%. The high positive and negative predictive values (PPVs and NPVs) of 97.2% and 99.7%, which the SC-OS showed in this study, further support the potential of the sample type and technique as an important clinical tool, especially in settings where conventional sputum collection may be challenging.

Of the TB positives, 58.3% were of medium bacterial load semi-quantitative readout, which is important in providing a near real-time estimate for infectiousness as well as maximum detection yield from symptomatic PCR-positive cases. The presence of a medium load could mean that many participants may be infectious, and note the importance of early diagnosis followed by commencement of treatment. With a lower number of 'very low' and 'trace' results (13.9% and 5.6%, respectively), the distribution suggests that SC-OS samples can capture biologically relevant numbers of TB bacteria, rendering this method feasible for TB screening, which is in agreement with the study by Luabeya et al., (23) that reported a similar finding.

The graph of the cycle threshold values of sputum samples and the SC-OS captured a high MTB yield of the SC-OS compared to sputum. This implies that SC-OS samples may be adequate and a suitable alternative sample for TB diagnosis. This is an important finding given the difficulty in obtaining sputum from some patient populations, such as children and

adults of advanced age or those with physical impairments and people living with HIV. Self-collection is convenient and may increase participation in TB screening programs because it provides patients with privacy and minimizes stigma, as well as providing patients with the opportunity to participate in their healthcare delivery

The findings from this study support inclusion of SC-OS as part of routine TB diagnostics, especially in hard-to-reach communities where collection and transportation of sputum in a cold chain is difficult. SC-OS has an advantage over sputum samples since it can be self-collected by patients and transported at room temperature to the testing laboratory, thereby eliminating the cold-chain transport system, which is hard to maintain in most cases, especially in hard-to-reach communities. This implementation could improve early diagnosis, reduce the spread of TB, and may lead to better TB control in Nigeria as well as other countries at comparable levels. Public health interventions are needed to educate communities on the advantages of SC-OS, reverse TB-related stigma, and impart knowledge regarding the appropriate ways individuals can collect samples.

The limitation of this study is that it was a cross-sectional study and therefore did not impact on the long-term outcome of TB patients in other centers. Furthermore, the low representation of younger people in this demographic composition may fail to reflect entirely on the TB burden among these age groups and thus underscores a case for tailored research. Future research should aim to understand the scalability of SC-OS methods among diverse populations and settings, as well as the durability of their effects on TB diagnostics and treatment compliance

Conclusion:

This study demonstrates that early morning self-collected oral swabs offer a highly effective and practical alternative to sputum for the early diagnosis of pulmonary TB by Xpert MTB, especially in hard-to-reach settings such as the remote communities in Karu. With sensitivity and specificity of 97.2% and 99.7%, the method performed remarkably well when compared with sputum Xpert MTB/RIF Ultra, capturing nearly all true positive cases and showing a strong capacity to detect *M. tuberculosis* even at lower bacterial loads. The strong concordance between sputum and oral swab results, alongside the high MTB yield reflected in the Ct values, highlights the diagnostic potential of this approach.

Beyond laboratory performance, the study also underscores the operational value of oral swabs in communities where sputum collection is difficult, cold-chain transport is challenging, and stigma affects care-seeking behaviors. By eliminating the need for invasive procedures, specialized personnel, and cold-chain logistics, self-collection can improve access to testing, increase community participation, and support earlier diagnosis and treatment initiation. Although further field validation and larger studies across different populations are needed, these findings provide promising evidence that self-collected oral swabs can strengthen TB case detection in Nigeria and similar high-burden regions. This approach can contribute meaningfully to national and global goals aimed at closing diagnostic gaps and improving the timeliness of care for individuals affected by tuberculosis.

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Contributions of authors:

TTE conceptualized the study, developed the study protocol, and made the first draft of the manuscript; PJ and AAA finalized and proofread the manuscript; TTE and SA perfor med the laboratory analysis; CU and TTE performed data analysis; JB reviewed the manuscript and provided overall guidance.

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Conflict of interest:

The authors declare that they have no financial or personal relationships that may

have inappropriately influenced them in writing this article.

AI use disclosure:

ChatGPT 3.5 and Grammarly were used during the early drafting of this manuscript. All intellectual contributions and final content are the result of the authors' work. The content was reviewed and edited by the authors, who take full responsibility for its accuracy

Data availability

The authors will make the data supporting the findings of this study available through the corresponding author upon reasonable request following our data sharing policy.

Disclaimer:

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of all the authors' affiliating institutions, nor the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP).

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Original Article **Open Access**

Detection of Dengue IgM and IgG among apparently healthy and sick individuals attending selected health facilities in rural and suburban areas of Osun State, Nigeria

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Abstract:

Background: Dengue fever, one of the viral infections that shares clinical features with malaria, is a reason for common misdiagnosis and under-reporting in resource-limited places. This cross sectional study was designed to determine the prevalence of Dengue virus (DENV) specific IgM and IgG antibodies among apparently healthy and sick individuals attending health facilites in selected rural and sub-urban areas in Osun State.
Methodology: This was a descriptive cross-sectional study of 164 randomly selected consenting participants visiting some healthcare facilities in selected localities of Osun State, Nigeria, between November 2024 and September 2025. Venous blood samples were collected from participants for the detection of DENV IgM and IgG using rapid diagnostic test kits following the manufacturers instructions. Data were collected with structured questionnaires and analysed using the Statistical Package for Social Sciences (SPSS) v23. Descriptive statistic was used for prevalence estimation, and Chi-square test was used to determine association between variables. Significance level was set at $p \leq 0.05$.
Results: The overall prevalence of DENV IgM among the participants was 7.3% (n=12/164) while that of DENV IgG was 14.6% (n=24/164). Co-detection of IgM and IgG occurred in 4.9% (n=8/164) of the participants. The prevalence of DENV IgM was highest among individuals aged 11-20 years (15.2%, n=5/33), followed by aged 61-70 years (9.1%, n=1/11) and 21-30 years (8.9%, n=5/58). Females had higher DENV IgM and IgG prevalence of 9.7% (n=11/113) and 18.6% (n=21/113) respectively, compared to males. Spatially, sub-urban dwellers had higher prevalence of DENV IgM (9.7%, n=6/62) and IgG (24.2%, n=15/62), compared to rural and urban dwellers. A significantly higher prevalence of DENV IgM (p=0.024) was observed among artisans (26.7%, n=4/15), compared to other occupational groups.
Conclusion: This study shows that Dengue virus infection is present in the population with evidence of both recent (IgM) and previous (IgG) infections, and there is the indication of re-infection, possibility of secondary infections or continuous reactions in the immune system, with the likelihood of pressure on the virus which may lead to mutations of any kind. This calls for surveillance with a wider coverage and laboratory empowerment to avoid misdiagnosis. A larger sample size and molecular analysis would give a better insight into the epidemiology of Dengue in the population.

Keywords: Dengue, detection, antibodies, seroprevalence, rural, sub-urban

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Détection des IgM et IgG anti-dengue chez des individus apparemment sains et malades fréquentant des établissements de santé sélectionnés dans les zones rurales et périurbaines de l'État d'Osun, au Nigéria

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Résumé:

Contexte: La dengue, une infection virale présentant des caractéristiques cliniques similaires à celles du paludisme, est souvent mal diagnostiquée et sous-déclarée dans les régions aux ressources limitées. Cette étude transversale

visait à déterminer la prévalence des anticorps IgM et IgG spécifiques du virus de la dengue (DENV) chez des personnes apparemment saines et malades fréquentant des établissements de santé dans des zones rurales et périurbaines sélectionnées de l'État d'Osun.
Méthodologie: Il s'agit d'une étude transversale descriptive menée auprès de 164 participants consentants, sélectionnés aléatoirement, consultant dans des établissements de santé de localités sélectionnées de l'État d'Osun, au Nigéria, entre novembre 2024 et septembre 2025. Des échantillons de sang veineux ont été prélevés chez les participants pour la détection des IgM et IgG anti-DENV à l'aide de tests de diagnostic rapide, conformément aux instructions du fabricant. Les données ont été recueillies à l'aide de questionnaires structurés et analysées avec le logiciel SPSS (version 23). Des statistiques descriptives ont été utilisées pour estimer la prévalence et le test du χ^2 pour déterminer l'association entre les variables. Le seuil de signification a été fixé à $p \leq 0,05$.
Résultats: La prévalence globale des IgM anti-DENV parmi les participants était de 7,3% (n=12/164), tandis que celle des IgG anti-DENV était de 14,6% (n=24/164). Une co-détection d'IgM et d'IgG a été observée chez 4,9% des participants (n=8/164). La prévalence des IgM anti-DENV était la plus élevée chez les personnes âgées de 11 à 20 ans (15,2%, n=5/33), suivie par celle des 61 à 70 ans (9,1%, n=1/11) et des 21 à 30 ans (8,9%, n=5/58). Les femmes présentaient une prévalence d'IgM et d'IgG anti-DENV plus élevée que les hommes, respectivement de 9,7% (n=11/113) et de 18,6% (n=21/113). Sur le plan spatial, la prévalence des anticorps anti-DENV IgM (9,7%, n=6/62) et IgG (24,2%, n=15/62) était plus élevée chez les habitants des zones périurbaines que chez ceux des zones rurales et urbaines. Une prévalence significativement plus élevée d'anticorps anti-DENV IgM (p=0,024) a été observée chez les artisans (26,7%, n=4/15) comparativement aux autres groupes professionnels.
Conclusion: Cette étude montre que l'infection par le virus de la dengue est présente dans la population, avec des preuves d'infections récentes (IgM) et anciennes (IgG). Elle suggère une possible réinfection, des infections secondaires ou des réactions continues du système immunitaire, susceptibles d'exercer une pression sur le virus et d'entraîner des mutations de toute nature. Ceci souligne la nécessité d'une surveillance plus étendue et d'un renforcement des capacités des laboratoires afin d'éviter les erreurs de diagnostic. Un échantillon plus important et une analyse moléculaire permettraient de mieux comprendre l'épidémiologie de la dengue au sein de la population.

Mots-clés: Dengue, détection, anticorps, séroprévalence, milieu rural, milieu périurbain

Introduction:

Dengue, an undifferentiated fever, asymptomatic or mild febrile disease, that can lead to dengue haemorrhagic fever (DHF), and/or Dengue shock syndrome (DSS) is a viral infection transmitted by *Aedes* spp mosquitoes (mainly *Aedes aegypti* and *Aedes albopictus*). The disease is broadly spread in tropical and subtropical areas of the world (1, 2). The prevalence of dengue has been increasing across the globe with the case reported to WHO rising to 14.6 million in 2024 after 505 430 cases in 2000, and has become endemic in over 100 countries (3). However, in Nigeria, it is endemic yet outbreaks are not properly documented and the actual burden of endemicity is not ascertained due to few or no systematic studies, limited data, insufficient diagnostic potential and misdiagnosis as malaria (4).

In Nigeria, arboviral infections have been reported to be confused with malaria because of the clinical similarity with malaria and the lack of regular surveillance (5). Dengue fever is also regarded as an issue in almost all the states of Nigeria, and one of the most frequent causes of fever diseases that are not correctly diagnosed (6). Dengue occurs both in urban and rural regions with more previous cases common in urban regions (7). The high rate of urbanization has made this arboviral disease an important issue of the urban population as it is aided by overpopulation, improper waste disposal, inadequate housing and garbage, car waste tires, tin, plastic, among others, which give the proper ecological condition that enables the breeding of urban populations of *Aedes* spp mosquitoes, the vector of the disease. The tropical and subtropical proliferation of urban populations of *Aedes* spp mosquitoes; the lack of effective control of vectors and prevention of infectious diseases have all made the disease to get perpetuated in the urban world (8,9).

The co-detection of specific antibodies to Dengue is crucial to the adaptation of dengue virus and the management of the disease. The aim of this study is to determine the prevalence of Dengue IgM and IgG and the co-detection of same among selected participants in rural and sub-urban localities in Osun State, Nigeria.

Materials and method:

Study design and setting:

This hospital-based study is a cross-sectional design involving apparently healthy individuals and those visiting the selected healthcare facilities for mild illness across the Ife Central, Ife East, and Ife North Local Government Areas in Osun State, Nigeria. The study areas have a tropical rainforest climate with high seasonal rainfall and mosquito breeding potential. A questionnaire to obtain socio-demographic data, behaviors and exposure to mosquito, and health history was administered to consenting individuals.

Ethical consideration:

Ethical approval for the study was obtained from the Health Research Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria. Informed consents of the participants were also obtained.

Study participants, sample size and selection:

The study participants were apparently healthy individuals and those visiting the selected healthcare facilities for mild illness regardless of age and sex. The sample size of 164 was calculated using the Leslie Kish formula ( ). The health facilities were randomly chosen across the Local Government Areas because of their relatively high patronage. The participants were selected using random sampling technique. Participants with serious health complications, physically weak, and those with severe infections, were excluded from the study.

Data collection:

A structured questionnaire was self-administered to each participant to collect socio-demographic and environmental information. Socio-demographic data collected included age, sex, occupation, educational level, and place of residence (urban, sub-urban or rural). Environmental and exposure-related data included proximity of residence to stagnant water bodies or refuse dumps, history of mosquito bites and outdoor occupational activities.

Sample collection and laboratory analysis:

About 3 ml of venous blood was collected aseptically from each participant. The blood samples were separated by centrifugation at 3,000 rpm for 15 minutes and the serum samples stored at -20°C until analyzed. The serum samples were serologically screened for Dengue virus IgM and IgG using Rapid Test Cassettes (Shandong Highplus Biotech, China). Results were recorded after 15 minutes as positive or negative according to the manufacturers instructions.

Statistical analysis:

The data collected with the questionnaires and laboratory procedures were analyzed using Statistical Package for Social Sciences (SPSS) v23. Descriptive statistics were used to determine prevalence, and Chi-square tests assessed associations between variables. Significance was set at $p \leq 0.05$.

Results

Sociodemographic characteristics of the study participants:

Of the 164 participants selected for the study, 51 (31.1%) were males while 113 (68.9 %) were females. The age group 21-30 years is the most represented (n=56, 34.1%) while age group 71-80 years is the least represented (n=2, 1.2%). The participants are mostly from semi-urban (37.8%, n=62), followed by rural (32.9%, n=54) and urban (29.3%, n=48) settlements, and were recruited mostly from primary health care facility (65.4%, n=107) and private facility (32.9%, n=54) while only 3 (1.8%) were from secondary healthcare facility (Table 1). The mean age of the 164 participants was 29.06 (SD = 18.17) years while the modal and median ages are 25 years and 24 years respectively.

Prevalence of Dengue IgM and IgG among the study participants:

Of the 164 participants, 12 (7.3%) were Dengue IgM positive, 24 (14.6%) were Dengue IgG positive, while 8 (4.9%) were positive for both Dengue IgM and IgG. Participants in the age group 11-20 years had the highest prevalence of DENV IgM (15.2%, n=5/33) followed by age group 21-30 years (8.9%, n=5/56), but the prevalence difference with respect to age was not statistically significant (p=0.448). The prevalence of DENV IgG was lowest in age group 0-10 years (5.3%, n=1/19) and highest in the age group 71-80 years (50.0%, n=1/2), but the prevalence difference was also not statistically significant with respect to age (p=0.537) (Table 1).

The prevalence of DENV IgM of 9.7% (n=11/113) for the female participants is higher than 2.0% (n=1/51) for the male participants, but the difference was not statistically significant (p=0.077). However, the prevalence of DENV IgG of 18.6% (n=21/113) for the female participants was significantly higher than 5.9% (n=3/51) for the male participants (p=0.033). Participants from suburban settlement had significantly higher prevalence of DENV IgG (24.2%, n=15/62) than participants from other settlements (p=0.021) but there was no significant difference in DENV IgM with respect to settlement type (p=0.658). Although participants from primary healthcare (HC) facility had higher prevalence of DENV IgM (10.3%, n=11/107) and DENV IgG (17.8%, n=19/107) than participants from secondary and private facilities, the prevalence difference was not statistically significant (p=0.135 and p=0.273 respectively) (Table 1).

Prevalence and distribution of the study participants positive for both DENV IgM/IgG with respect to location:

The prevalence and distribution of the study participants positive for both Dengue IgM

Table 1: Prevalence and distribution of dengue IgM and IgG antibodies among participants in Ile-Ife and environs

Characteristics	Participants (n)	DENV IgM			DENV IgG		
		+ve (%)	-ve (%)	*p* value	+ve (%)	-ve (%)	*p* value
Age group (years)	0-10 (n=19)	0	19 (100.0)	0.448	1 (5.3)	18 (94.7)	0.537
	11-20 (n=33)	5 (15.2)	28 (84.8)		6 (18.2)	27 (81.8)	
	21-30 (n=56)	5 (8.9)	51 (91.1)		6 (10.7)	50 (89.3)	
	31-40 (n=22)	1 (4.5)	21 (95.5)		4 (18.2)	18 (81.8)	
	41-50 (n=8)	0	8 (100.0)		1 (12.5)	7 (87.5)	
	51-60 (n=13)	0	13 (100.0)		2 (15.4)	11 (84.6)	
	61-70 (n=11)	1 (9.1)	10 (90.9)		3 (27.3)	8 (72.7)	
	71-80 (n=2)	0	2 (100.0)		1 (50.0)	1 (50.0)	
	Total (n=164)	12 (7.3)	152 (92.7)		24 (14.6)	140 (85.4)	
Sex	Male (n=51)	1 (2.0)	50 (98.0)	0.077	3 (5.9)	48 (94.1)	*0.033
	Female (n=113)	11 (9.7)	102 (90.3)		21 (18.6)$^{+}$	92 (81.4)	
	Total (n=164)	12 (7.3)	152 (92.7)		24 (14.6)	140 (85.4)	
Settlement type	Rural (n=54)	3 (5.6)	51 (94.4)	0.658	6 (11.1)	48 (88.9)	*0.021
	Sub-urban (n=62)	6 (9.7)	56 (90.3)		15 (24.2)$^{+}$	47 (75.8)	
	Urban (n=48)	3 (6.3)	45 (93.8)		3 (6.3)	45 (93.8)	
	Total (n=164)	12 (7.3)	152 (92.7)		24 (14.6)	140 (85.4)	
Health facility	Primary HC (n=107)	11 (10.3)	96 (89.7)	0.135	19 (17.8)	88 (82.2)	0.273
	Secondary HC (n=3)	0	3 (100. 0)		0	3 (100.0)	
	Private facility (n=54)	1 (1.9)	53 (98.1)		5 (9.3)	49 (90.7)	
	Total (n=164)	12 (7.3)	152 (92.7)		24 (14.6)	140 (85.4)	

*=Statistically significant; $^{+}$=siginficantly higher prevalence; n=nunber of participant; DENV=Dengue virus

Table 2: Distribution of participants positive for both dengue IgG and IgM with respect to locations

Locations	Dengue IgM	Dengue IgG		Total
		Negative	Positive	
Akarabata (n=5)	Negative	5	0	5
	Positive	0	0	0
Edunabon (n=12)	Negative	11	1	12
	Positive	0	0	0
Enuwa (n=20)	Negative	17	0	17
	Possitive	0	3 (15.0%)	3
Ipetumodu (n=8)	Negative	8	0	8
	Positive	0	0	0
Modakeke (n=17)	Negative	10	4	14
	Positive	0	3 (17.6%)	3
OAU Campus (n=43)	Negative	38	2	40
	Positive	2	1 (2.3%)	3
Okookan (n=1)	Negative	1	0	1
	Positive	0	0	0
Sabo (n=1)	Negative	1	0	1
	Positive	0	0	0
Sooko (n=16)	Negative	11	3	14
	Positive	2	0	2
Wanikin (n=25)	Negative	23	1	24
	Positive	0	1 (4.0%)	1
Yakooyo (n=16)	Negative	11	5	16
	Positive	0	0	0

and IgG according to location is shown in Table 2. The prevalence of DENV IgG/IgM was 17.6% (n=3/17) for participants from Modakeke, 15% (n=3/20) for Enuwa, 4.0% (n=1/25) for Wani-

kin, and 2.3% (n=1/43) for OAU Campus. With respect to sex of the 8 participants positive for both DENV IgG and IgM, 6 were females (75%); 3 (37.5%) from Enuwa and 3 (37.5%) from Modakeke, while 2 were males (25.0%). However, the prevalence of both DENV IgM and IgG of 3.9% (n=2/51) in the males is not significantly different from 5.3% (6/113) in the females (p=1.000). All the participants positive for both DENV IgM and IgG were from the sub-urban study areas.

Predisposing factors for DENV infection:

In Table 3 on predisposing factors to DENV infection, occupation of the participants was significantly associated with DENV IgM positivity (p-value of 0.024). The prevalence of DENV IgM (recent Dengue virus infection) was significantly higher among artisans (26.7%, n= 4/15) compared to traders (3.7%, n=2/54), civil servants (5.9%, n=1/17) and unemployed (6.4%, n=5/78) (p=0.024)

Discussion:

This study shows that there are recent and past Dengue virus infections in rural and suburban regions of Osun State, Nigeria as indicated by the presence of dengue IgM and IgG antibodies in the selected participants. The fact that there is a higher prevalence of IgG (14.6%) over IgM (7.3%) indicates that a high proportion of the population in the study setting had prior exposure to the Dengue virus thus, suggesting continuous Dengue transmission in the study area. This situation is likely to be similar to other regions of Nigeria and sub-Saharan Africa, where the transmission of Dengue is persistent, neglected, and hence under-reported because of insufficient routine diagnostics and likely misdiagnosis with malaria or other febrile diseases (9).

The co-detection of dengue IgM and IgG antibodies in the study participants is epidemiologically important because it indicates secondary infections occurring in people who were previously exposed to it. The secondary dengue infection has been reported to enhance the possibility of having severe clinical manifestations because of antibody-dependent enhancement, which has been well reported in dengue pathogenesis (10). The same patterns of co-detection were reported in other studies in Nigeria and other endemic countries, where people with a history of exposure are prone to infection with other dengue serotypes, hence leading to disease severity and persistence of infection (11,12). Molecular analysis will help to ascertain the predominant serotypes and hence the disease could be better managed.

The high prevalence of Dengue IgM and IgG among the age group of 11-20 years indicates that young adults and more active groups of the population might be at a higher risk of Dengue virus infection. It is also worth noting that DENV IgG prevalence increased progressively from the 41-50 to 71-80 years. Reasons for this may be due to their profession, travel history, and behavioural patterns which have exposed them to the daytime feeding *Aedes* mosquitoes.

Table 3: Predisposing factors to Dengue virus infections among the study participants

Characteristics	**Responses**	**DENV IgM**		***p* value**
		+ve (%)	**-ve (%)**	
Time Indoor (n=145)**	Around 6pm	3 (8.1)	34 (91.9)	0.690
	Around 8pm	6 (8.6)	64 (91.4)	
	Around 10pm	3 (7.9)	35 (92.1)	
Recent Mosquitoes Bite (n=144)**	No	2 (8.0)	23 (92.0)	0.692
	Yes	5 (5.1)	93 (94.9)	
	Not sure	2 (9.5)	19 (90.5)	
Residence infested with Mosquitoes (n=144)**	No	2 (5.6)	34 (94.4)	0.500
	Yes	7 (7.6)	85 (92.4)	
	Yes, but seasonal	0	16 (100.0)	
Occupation (n=164)	Trading	2 (3.7)	52 (96.3)	*0.024
	Artisan	4 (26.7)$^{+}$	11 (73.3)	
	Civil Servant	1 (5.9)	16 (94.1)	
	Not Employed	5 (6.4)	73 (93.6)	
Live close to Stagnant water (n=164)	Yes	7 (13.0)	47 (87.0)	0.52
	No	5 (4.5)	105 (95.5)	

*Statistical significance; **Based on number of participants who responded to the questionnaires; $^{+}$significantly higher prevalence

The increasing prevalence of the IgG among the elderly ones may be due to their previous exposure and may be suggesting an increased Dengue IgG prevalence in the future since younger ones have high DENV IgM prevalence now. Similar results have been documented in the northern Nigeria and southeast Asia where adolescents and young adults showed greater prevalence of acute Dengue virus infection because they were more mobile and exposed to the environment (3,11).

Although the prevalence of DENV IgM (9.7%) and IgG (18.6%) are higher for the female participants in this study, there may be no special reason for this high prevalence but it may be as a result of female-related roles and behaviors, which expose them to mosquito bites. Women tend to be more engaged in water handling and waste management activities in most African environments thus predisposing them to visiting breeding sites of the mosquito vectors. The reason for the prevalence may therefore be a context-specific phenomenon (9). More studies about the immune response may also be useful to give further explanation for this difference. The transmission patterns are likely to have been aided by environmental and socio-economic factors.

Problems that are common in rural and suburban areas in Osun State include poor waste disposal mechanisms, poor drainage, and use of water storage containers, which are favorable breeding environments for the *Aedes* mosquitoes. These results are consistent with earlier reports claiming that environmental sanitation and water management practices are also among the most important determinants of dengue spread in the endemic areas (13,14). Sub-urban environments tolerate a higher level of mosquitoes because of environmental conditions like open water containers, temperature, and humidity which favor larvae growth and survival of vectors (15).

The co-detection of IgM with IgG suggests that as long as active transmission takes place, a significant percentage of infections might remain unrecognized as febrile diseases, in many resource-deficient facilities, are often treated presumptively as malaria without laboratory verification thus leading to the underestimation of real burden of this disease (16). The growing epidemiological significance of sub-urban regions as crucial areas of Dengue virus transmission was further substantiated by this study with a higher prevalence of the Dengue IgM in the suburban areas. Moreover, spatial epidemiological empirical evidence shows that hot-spots of Dengue transmission are often related to rapidly growing and sub-urban areas whose human mobility, housing configurations, and ecological situations interact and perpetuate transmission cycles (17).

Rising urbanization and environmental transformation have been cited as one of the biggest causes of arboviral pathology emergence such as dengue within the transitional urban-sub-urban zones in sub-Saharan Africa (18). Also, studies in West Africa have proved that peri-urbanization promotes the continued transmission of Dengue as it predisposes humans to develop closer interactions with vectors besides the environment being more favorable (19). This change is a cause of the necessity to have inclusive approaches to public health, which consider urban and non-urban environments.

This study supports the significance of serological surveillance in the detection of recent and previous infections. There is possibility that the observed higher seroprevalence in sub-urban residents in this study indicates an interaction between ecological suitability, the abundance of vectors, and human patterns of exposure, which stress the importance of specific interventions targeting the control of vectors and the environment in high-risk regions. The co-detection of Dengue IgM and IgG antibodies buttresses the risk of second-line infections and a possible severe development of the disease. Enhancing diagnostic capacity, environmental sanitation, and specific interventions in the field of the implementation of vector control are the necessary measures to reduce the burden of dengue in Osun State and other such facilities.

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Contributions of authors:

AOA conceived the idea, was involved in the laboratory analysis and the reading and correction of the manuscript; OAA was involved in sample collection, laboratory analysis and the writing of the manuscript.

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Original Article **Open Access**

Prevalence and risk factors associated with intestinal parasitic infections among adults in rural Itasy region of Madagascar

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Abstract:

Background: Intestinal parasitic infections (IPIs) are a major public health burden in rural Madagascar, but adult data are scarce. We aimed to estimate the prevalence of IPI and determine associated factors among adults attending a referral hospital in the Itasy region of Madagascar.
Methodology: A hospital-based cross-sectional study was conducted between June and August 2023 on consecutively enrolled adults (≥18 years) at the Itasy Regional Referral Hospital. A single fresh stool sample per participant was examined by direct wet mount. Sociodemographic, clinical and behavioural data were collected. Factors associated with IPI prevalence were assessed using bivariate and multivariate logistic regression.
Results: Among 101 participants (57 women, median age 37.8 years), IPI prevalence was 34.7% (n=35/101). The most frequent parasites were *Entamoeba histolytica/dispar* (37.1%), *Schistosoma mansoni* (34.3%) and *Ascaris lumbricoides* (31.4%). In multivariate analysis, low socioeconomic status (aOR = 8.90), proximity to rice fields or untreated water (aOR = 6.80), and farming occupation (aOR = 5.60) were associated with high IPI prevalence. Deworming within 12 months (aOR = 0.22) and safe food handling (aOR = 0.05) were associated with low IPI prevalence.
Conclusion: IPIs affected more than one-third of adults in this rural Malagasy hospital sample. Low socioeconomic status, farming, and living near untreated water were strong correlates, while recent deworming and safe food handling were associated with lower risk. Because only one stool sample without concentration was used, the true prevalence is likely underestimated. Larger community-based studies with multiple samples and concentration techniques are needed.

Keywords: Intestinal parasites; Prevalence; Risk factors; Madagascar

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Prévalence et facteurs de risque associés aux infections parasitaires intestinales chez les adultes de la région rurale d'Itasy à Madagascar

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Résumé:

Contexte: Les infections parasitaires intestinales (IPI) représentent un problème majeur de santé publique en milieu rural à Madagascar, mais les données concernant les adultes sont rares. Cette étude visait à estimer la prévalence des IPI et à déterminer les facteurs associés chez les adultes consultant un hôpital de référence de la région d'Itasy, à Madagascar.
Méthodologie: Une étude transversale en milieu hospitalier a été menée entre juin et août 2023 auprès d'adultes (≥ 18 ans) recrutés consécutivement à l'hôpital régional de référence d'Itasy. Un échantillon de selles fraîches par participant a été examiné par examen microscopique direct. Des données sociodémographiques, cliniques et comportementales ont été recueillies. Les facteurs associés à la prévalence des IPI ont été évalués par régression logistique bivariée et multivariée.
Résultats: Parmi les 101 participants (57 femmes, âge médian : 37,8 ans), la prévalence des IPI était de 34,7% (n=35/101). Les parasites les plus fréquemment retrouvés étaient *Entamoeba histolytica/dispar* (37,1%), *Schistosoma mansoni* (34,3%) et *Ascaris lumbricoides* (31,4%). L'analyse multivariée a révélé qu'un faible statut socio-économique (ORa = 8,90), la proximité de rizières ou de points d'eau non traités (ORa=6,80) et l'exercice de la profession agricole (ORa = 5,60) étaient associés à une forte prévalence d'infections parasitaires intestinales (IPI). Un traitement vermifuge au cours des 12 derniers mois (ORa=0,22) et le respect des bonnes pratiques d'hygiène alimentaire (ORa=0,05) étaient associés à une faible prévalence d'IPI.
Conclusion: Les IPI ont touché plus d'un tiers des adultes de cet échantillon hospitalier rural malgache. Un faible statut socio-économique, l'exercice de la profession agricole et la proximité de points d'eau non traités étaient des facteurs fortement corrélés, tandis qu'un traitement vermifuge récent et le respect des bonnes pratiques d'hygiène alimentaire étaient associés à un risque moindre. L'utilisation d'un seul échantillon de selles non concentré suggère une sous-estimation de la prévalence réelle. Des études communautaires de plus grande envergure, avec des prélèvements multiples et des techniques de concentration, sont nécessaires.

Mots-clés: Parasites intestinaux; Prévalence; Facteurs de risque; Madagascar

Introduction:

Intestinal parasitic infections (IPIs) remain a major global public health burden, particularly in low- and middle-income countries where access to clean water, adequate sanitation, and hygiene infrastructure is limited. Worldwide, approximately 3.5 billion people are affected by IPIs, with the World Health Organization (WHO) estimating that 1.5 billion individuals are infected with soil-transmitted helminths alone (1). In sub-Saharan Africa, IPIs are responsible for considerable morbidity, including chronic diarrhea, malnutrition, anaemia, and impaired cognitive development, and contribute to approximately 155,000 deaths annually (2).

Madagascar, one of the poorest countries in sub-Saharan Africa, carries a disproportionately high burden of IPIs. National reports indicate that geohelminth infections affect 111 of 114 districts, schistosomiasis is endemic in 107 districts, and *Taenia solium* taeniasis has been reported in 54 districts (3,4). Despite the introduction of a national neglected tropical disease (NTD) master plan in 2014, IPIs remain highly prevalent. For example, a study in two disadvantaged neighbourhoods of Antananarivo found that 96.3% of children aged 2-5 years harboured at least one intestinal parasite, with *Giardia intestinalis* (79.5%), *Ascaris lumbricoides* (68.3%) and *Trichuris trichiura* (68.0%) being the most common (5).

Similarly, in Mahajanga on the west coast, a prospective study of 265 adults found an overall IPI prevalence of 77%, with protozoa being the most common group (72.8%) (6,7). A 1996-1997 hospital-based study in the same region reported that 67.6% of stool samples contained at least one parasite, with protozoa (47.7%) predominating over nematodes (23.4%) (7). In rural areas, IPIs are closely linked to agricultural activities, exposure to contaminated water bodies, and poor sanitation. The Itasy region, located in the central highlands approximately 90 km west of Antananarivo, is characterized by numerous lakes, rice paddies, and irrigation canals that provide ideal breeding grounds for *Biomphalaria* snail, the intermediate host of *Schistosoma mansoni.* The region is known to be highly endemic for intestinal schistosomiasis, with one study reporting a prevalence of 59.5% among adults (8). A systematic review of schistosomiasis in Madagascar estimated a pooled prevalence of 49.3% (95% CI: 28.7-72%) for intestinal schistosomiasis (9).

Moreover, the Itasy region faces significant socioeconomic challenges. A 2013 monograph reported that majority of the population relies on subsistence agriculture, with limited access to piped water, latrines, and health services (10). Despite these alarming figures, most national control efforts, including mass drug administration (MDA) campaigns have focused primarily on school-aged children, follow-

ing the WHO recommendations for preventive chemotherapy. Adults, particularly those engaged in agriculture and living in rural areas, are often overlooked despite their constant exposure to contaminated soil and water, their role as reservoirs of transmission, and their potential to reinfect children within households (9). Few studies have specifically examined IPI burden and associated risk factors among adults in Madagascar, and no recent data are available for the Itasy region (4).

Given this knowledge gap, the present study was designed to; (i) determine the crude prevalence of intestinal parasitic infections among adults attending the Itasy Regional Referral Hospital (CHRR Itasy); (ii) describe the spectrum of parasite species involved; (iii) identify sociodemographic, clinical, and behavioral factors associated with IPI positivity; and (iv) propose evidence-based recommendations for improving screening and management in this rural setting.

Materials and method:

Study design and setting:

A descriptive analytical cross-sectional study was conducted at the Itasy Regional Referral Hospital (CHRR Itasy) in Miarinarivo, the capital of the Itasy region, Madagascar. The hospital is located approximately 89 km west of Antananarivo along National Road 1. It serves a predominantly rural population engaged in agriculture (mainly rice cultivation) and fishing. The Itasy region is characterized by numerous lakes (including Lake Itasy), extensive rice paddies, and irrigation canals, which create ideal conditions for the transmission of water-borne parasites, particularly *S. mansoni* (10). The study was conducted over a period of three months (June to August 2023).

Study population and method of sampling:

All adults (aged ≥18 years) attending outpatient clinics or hospitalized at CHRR Itasy during the study period were eligible. Participants were recruited consecutively. Inclusion criteria were: (i) age ≥18 years; (ii) attendance at outpatient clinic or hospitalization during the study period; (iii) provision of written informed consent; and (iv) agreement to provide a stool sample for examination. Exclusion criteria were: (i) refusal to participate; (ii) failure to provide a stool sample; (iii) age <18 years.

A total of 215 eligible adults were approached during the three-month study period. Of these, 101 provided informed consent and a stool sample and were included in the analysis.

Data collection and instrument:

A structured questionnaire was developed based on a literature review and face-validated by a panel of three biologists. It was then pretested on 20 patients (not included in the final sample) at the Centre Hospitalier de Soavinandriana (CENHOSOA), Antananarivo. Based on the pretest, ambiguous questions were rephrased and redundant items were removed. The final version was administered by trained research assistants

Data collected included; (i) sociodemographic information: age, sex, marital status, education level, occupation, monthly income (< or ≥ the national minimum wage of 250,000 Malagasy Ariary), place of residence (proximity to rice fields or untreated water sources), type of housing (durable, semi-durable, or precarious), number of children in charge, and household crowding (>3 persons per bedroom); (ii) socioeconomic status, assessed using the validated Socioeconomic Status Composite Scale (SES-C), which combines seven weighted criteria (education, occupation, income, housing type, housing location, number of dependent children, and household crowding) into a composite score. Scores ≤10 were classified as low socioeconomic status, and scores ≥11 as acceptable/comfortable (11); (iii) clinical history: history of diarrhea, dysentery, abdominal pain, constipation; recurrence of digestive symptoms (≥3 episodes in the preceding 3 months); history of medical consultation or self-medication for digestive symptoms; similar symptoms in household members; (iv) preventive practices: handwashing frequency (≥3 times/day), use of clean water and soap for personal hygiene, washing food before consumption, safe food handling (defined as proper cooking, storage and protection of food), deworming during the previous 12 months, systematic deworming every 6 months, and any history of prior stool examination; and (v) behavioral and environmental exposures: eating outside the home; consumption of pork, beef, or fish; defecation in open areas; use of adequate latrines; shared toilets with multiple families; bathing in natural untreated water bodies (lakes, rivers); non-occupational activities in rice fields; and laundry in untreated water sources.

Stool collection and laboratory examination:

Each participant was provided with a clean, dry, leak-proof container labeled with a unique code. Participants were instructed to collect fresh stool sample (approximately 5g or the size of a thumb) in the morning and bring to

the hospital laboratory within one hour of collection. Upon receipt, samples were processed immediately.

Macroscopic examination was performed to assess consistency (formed, pasty, liquid), color, and the presence of visible elements (mucus, blood, adult worms, proglottids). Microscopic examination was performed using the direct wet mount method. Approximately 2 mg of stool sample was mixed with a drop of normal saline on a glass slide, covered with a coverslip, and examined under the light microscope (objective ×10 and ×40). A second preparation was made with Lugol's iodine to enhance visualization of protozoan cysts and nuclei (12). A positive result was defined as the presence of at least one parasite stage (trophozoite, cyst, egg, or larva) on either preparation. Parasite identification was based on standard morphological criteria. Parasite load was categorized as low (1 parasite per microscopic field), moderate (2 parasites per field), or high (≥3 parasites per field or described as "numerous").

Statistical analysis:

Data were entered into Microsoft Excel 2019 and double-checked for accuracy. Statistical analyses were performed using EPI INFO version 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS version 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Categorical variables were summarized as frequencies and percentages; continuous variables were summarized as means with standard deviations or medians with ranges, as appropriate.

Bivariate associations between IPI positivity and potential risk factors were assessed using Pearson's Chi-square test or Fisher's exact test, as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Variables with a *p*-value <0.20 in bivariate analysis were considered for inclusion in the multivariate model, together with age and sex (as a priori confounders).

Given the sample size (n=101) and the number of IPI-positive participants (n=35), we limited the multivariate model to a maximum of four variables to avoid overfitting (rule of ten events per variable). Variables with $p<0.20$ in bivariate analysis were considered for inclusion. Because age and sex are known potential confounders, we initially forced them into the model; they were not significantly associated with IPI and did not change the effect estimates of the other variables by >10%, so we removed them from the final reduced model.

A forward stepwise logistic regression was performed including the following variables: proximity to water bodies, farming occupation, low socioeconomic status, deworming within 12 months, and safe food handling. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were calculated. The Hosmer-Lemeshow test was used to assess goodness-of-fit. Statistical significance was set at $p<0.05$.

Ethical consideration:

Administrative authorization was obtained from the Director of CHRR Itasy and the Head of the Laboratory Department. The study was conducted in accordance with the Declaration of Helsinki. All participants provided writ-ten informed consent (or witnessed oral consent for participants with limited literacy) before enrollment. Participants were informed that refusal to participate would not affect their medical care. Stool examinations were provided free of charge, and participants with positive results received a free medical prescription (medications to be purchased at the participant's expense). Anonymity was maintained by using unique codes instead of names on all data collection forms and laboratory records.

Results

Study population characteristics:

A total of 101 adults were included in the analysis (44 men, 57 women), giving a sex ratio of 0.77. The median age was 37.8 years (range 18–>60 years). The majority of participants (54.5%, 55/101) lived in immediate proximity to rice fields or untreated water sources. Farming was the most common occupation (61.4%, 62/101). Low socioeconomic status (SES-C score ≤10) was present in 75 participants (74.3%, 75/101). Table 1 summarises the sociodemographic characteristics of the study population.

Prevalence of intestinal parasitic infections:

Overall, 35 participants (34.7%) tested positive for at least one intestinal parasite. Helminths (metazoans) were more frequent (80.0%, 28/35) than protozoa (40.0%, 14/35). Monoparasitism was observed in 30 participants (85.7%, 30/35) and multiparasitism in 5 participants (14.3%, 5/35).

Types of parasites identified:

The most common parasites were *Entamoeba histolytica/dispar* (37.1%, 13/35), *S. mansoni* (34.3%, 12/35), and *A. lumbricoides* (31.4%, 11/35). Other parasites identified included *Giardia lamblia* (14.3%, 5/35) and *Pen-*

tatrichomonas hominis (2.9%, 1/35). Table 2 presents the frequency of each parasite species. Among co-infections, the following associations were observed; *E. histolytica/dispar*+ *G. lamblia* (n=2), *E. histolytica/dispar*+*A. lumbricoides* (n=1), *S. mansoni* + *A. lumbricoides* (n=1), and *S. mansoni* + *P. hominis* + *A. lumbricoides* (n=1).

Table 1: Sociodemographic characteristics of study participants

Characteristics	Number	Percentage
Age group (years)		
18–24	23	22.8
25–59	65	64.4
≥60	13	12.9
Sex		
Male	44	43.6
Female	57	56.4
Proximity to water bodies/rice fields		
Yes	55	54.5
No	46	45.5
Occupation		
Farmer	62	61.4
Vendor	11	10.9
Housewife	7	6.9
Student	11	10.9
Other (civil servant, etc.)	10	9.9
Socioeconomic status (SES-C)		
Low (score ≤10)	75	74.3
Acceptable/comfortable (score ≥11)	26	25.7

Table 2: Frequency of specific intestinal parasites among infected adults

Parasite species	Number	Percentage
Entamoeba histolytica/dispar	13	37.1
Schistosoma mansoni	12	34.3
Ascaris lumbricoides	11	31.4
Giardia lamblia	5	14.3
Pentatrichomonas hominis	1	2.9

A single participant could harbour more than one parasite (co-infection).
The sum of percentages exceeds 100% due to multiparasitism.

Bivariate analysis:

Bivariate analyses of potential risk factors and IPI positivity are shown in Table 3. Low socioeconomic status (cOR=20.7; 95% CI: 2.70–158.2; p=0.001), proximity to water bodies (100% of infected participants vs. 30.3% of uninfected; p<0.001), and farming occupation (cOR=12.8; 95% CI 3.90–42.1; p<0.001) were strongly associated with increased odds of IPI.

Diarrhoea or dysentery was reported by 91.4% (32/35) of infected participants and was associated with four-fold odds of IPI (cOR =4.00; 95% CI: 1.09–14.70; p=0.038). Protective factors in bivariate analysis included deworming within the past 12 months (cOR= 0.19; 95% CI: 0.08–0.46; p<0.001), systematic deworming every 6 months (cOR=0.26; 95% CI: 0.09–0.71; p=0.008), safe food handling (cOR=0.08; 95% CI: 0.02–0.36; p<0.001) handwashing with soap (cOR=0.26; 95% CI: 0.10–0.64; p=0.003), and use of clean water for hygiene (cOR=0.26; 95% CI: 0.11–0.62; p=0.002).

Behavioural exposures such as bathing in natural untreated water, laundry in untreated water, and open defecation were also associated with higher IPI odds (Table 3). Proximity to water bodies was reported by all infected participants (35/35, 100%) compared to 30.3% (20/66) of uninfected participants (p< 0.001, Fisher's exact test). This perfect separation prevented the calculation of crude odds ratio but indicates a very strong association.

Multivariate analysis:

As shown in Table 4, after adjustment in multivariate analysis, low socioeconomic status remained strongly associated with high prevalence of IPI (aOR=8.90; 95% CI: 1.90–41.5; p=0.005), as did proximity to water bodies (aOR=6.80; 95% CI: 2.10–21.9; p<0.001) and farming occupation (aOR=5.60; 95% CI: 1.70–18.5; p=0.004). Deworming within the past 12 months (aOR=0.22; 95% CI: 0.08–0.62; p=0.004) and safe food handling (aOR= 0.05; 95% CI: 0.01–0.40; p=0.003) remained significantly associated with low prevalence of IPI. The association of diarrhoea or dysentery with high IPI in bivariate analysis was no longer statistically significant after adjustment in multivariate logistic analysis (aOR=3.20; 95% CI: 0.95–10.8; p=0.06).

Clinical and preventive practice gaps:

Among infected participants, despite 91.4% (32/35) reporting diarrhoea or dysentery, only 22.9% (8/35) had consulted a health professional for their symptoms, and only 14.3% (5/35) had ever undergone a prior stool examination. The majority of infected participants (74.3%, 26/35) had not received deworming in the preceding 12 months.

Table 3: Bivariate analysis of factors associated with intestinal parasitic infection (IPI) positivity among adults

Variables	IPI+ (n=35)	IPI- (n=66)	cOR (95% CI)	*p* value
Sociodemographic characteristics				
Age ≥ 40 years	16 (45.7)	30 (45.5)	1.01 (0.45–2.28)	0.98
Female sex	19 (54.3)	38 (57.6)	0.87 (0.39–1.97)	0.75
Low socioeconomic status (SES-C ≤10)	34 (97.1)	41 (62.1)	20.7 (2.70–158.2)	0.001
Proximity to water bodies (rice fields/lakes/rivers)	35 (100.0)	20 (30.3)	NC	<0.001
Farming occupation	32 (91.4)	30 (45.5)	12.8 (3.90–42.1)	<0.001
No formal education	24 (68.6)	31 (47.0)	2.46 (1.07–5.67)	0.035
Household crowding (>3 persons/bedroom)	22 (62.9)	35 (53.0)	1.49 (0.66–3.37)	0.34
Clinical symptoms				
Diarrhoea or dysentery	32 (91.4)	44 (66.7)	4.00 (1.09–14.70)	0.038
Abdominal pain	28 (80.0)	46 (69.7)	1.74 (0.67–4.51)	0.26
Constipation	17 (48.6)	28 (42.4)	1.28 (0.57–2.86)	0.55
Preventive practices				
Deworming within 12 months	9 (25.7)	42 (63.6)	0.19 (0.08–0.46)	<0.001
Systematic deworming every 6 months	6 (17.1)	29 (43.9)	0.26 (0.09–0.71)	0.008
Safe food handling	2 (5.7)	28 (42.4)	0.08 (0.02–0.36)	<0.001
Handwashing ≥3 times/day with soap	8 (22.9)	35 (53.0)	0.26 (0.10–0.64)	0.003
Use of clean water and soap for hygiene	10 (28.6)	40 (60.6)	0.26 (0.11–0.62)	0.002
Behavioural exposures				
Bathing in natural untreated water	28 (80.0)	39 (59.1)	2.77 (1.08–7.10)	0.034
Laundry in untreated water	25 (71.4)	30 (45.5)	3.00 (1.26–7.16)	0.013
Open defecation	22 (62.9)	28 (42.4)	2.30 (1.02–5.22)	0.045

Chi-square or Fisher's exact test; NC = not calculable due to zero cell count in one group; IPI+ = participants with at least one intestinal parasitic infection; IPI− = participants without intestinal parasitic infection. cOR = Crude odds ratio. CI=Confidence interval. Note: Proximity to water bodies showed 100% exposure among IPI-positive participants, therefore a crude OR could not be calculated (zero cell in unexposed/IPI+ group)

Table 4: Multivariate logistic regression analysis of factors associated with intestinal parasitic infection – final reduced model

Variables	Reference category	aOR (95% CI)	*p*-value
Low socioeconomic status (SES-C ≤10)	Acceptable/comfortable SES (≥11)	8.90 (1.90–41.5)	0.005
Proximity to water bodies	No proximity	6.80 (2.10–21.9)	<0.001
Farming occupation	Non-farming occupation	5.60 (1.70–18.5)	0.004
Deworming within 12 months	No deworming	0.22 (0.08–0.62)	0.004
Safe food handling	Unsafe food handling	0.05 (0.01–0.40)	0.003

Final reduced model refers to the model obtained after forward stepwise selection (entry $p<0.20$, stay $p<0.05$) among variables with $p<0.20$ in bivariate analysis, given the limited number of events (n=35). aOR = Adjusted odds ratio; CI=Confidence internal
Age and sex were initially forced as a priori confounders but were non-significant and did not alter effect estimates; they were removed from the final model. Hosmer-Lemeshow test p = 0.68, indicating good model fit

Discussion:

This study provides one of the few recent estimates of IPI prevalence among adults in a rural area of Madagascar. The observed prevalence of 34.7% suggests that IPIs remain a substantial public health problem in the Itasy region, affecting more than one-third of adults attending the regional referral hospital. However, because we used only one stool sample without concentration, this is likely a minimum estimate. The prevalence is lower than the 77.0% reported in Mahajanga using molecular methods (6) and the 67.6% reported earlier in Mahajanga with concentration techniques (7). This difference is expected given our use of single-sample direct microscopy without concentration, which underestimates true prevalence especially for protozoa. Our prevalence is comparable to the 26% reported in Biskra, Algeria (13), and to the 28% reported in a recent study from southern Algeria (14).

The predominance of *E. histolytica/ dispar* (37.1%), *S. mansoni* (34.3%) and *A. lumbricoides* (31.4%) aligns with the known epidemiology of IPIs in Madagascar. In the Antananarivo study of children aged 2-5 years of age, *G. intestinalis* (79.5%), *A. lumbricoides* (68.3%), and *Trichuris trichiura* (68.0%) were most prevalent, with *E. histolytica* detected in 15.8% of stunted children (5). In Mahajanga, Greigert et al., (6) reported a wide panel of parasites with protozoa (72.8%) dominating, including *Blastocystis* sp, *Dientamoeba fragilis Entamoeba coli*, and *G. intestinalis*.

The high prevalence of *S. mansoni* in

our study (34.3%) is particularly noteworthy. Madagascar has the fifth-highest burden of schistosomiasis globally, with 106 of 113 districts considered endemic (9). A systematic review estimated a pooled prevalence of 49.3% (95% CI: 28.7-72%) for intestinal schistosomiasis (9). In the Itasy region, historical data from Ampefy on Lake Itasy reported prevalence rates exceeding 70% in the early 1990s when control programmes were interrupted. More recently, a study in Andina and Ankazomborona reported a prevalence of 59.5% for *S. mansoni* among adults (8). The abundance of lakes, rice paddies, and irrigation canals in Itasy provides ideal breeding grounds for *Biomphalaria* snails, and agricultural activities ensure frequent water contact, perpetuating transmission (10).

The observation that all IPI-positive participants lived near rice fields or untreated water sources, corresponding to nearly sevenfold increased risk after adjustment (aOR= 6.80), underscores the critical role of environmental exposure. This is consistent with studies from Mauritania, where children living in humid regions had significantly higher IPI prevalence (33.4%) (15), and from Vietnam, where peri-urban farmers living near polluted water had a 5.8-fold increased risk (aOR=5.8; 95% CI: 2.5-13.7) (16). The strong association with agricultural occupation (aOR=5.60; 91.4% of infected participants were farmers) is also consistent with the international literature. In Ethiopia, farmers had a 1.85-fold higher risk of geohelminth infection compared to non-farmers (17), and in Pakistan, farmers using wastewater irrigation had an OR of 31.4 for hookworm infection (18). The Itasy region's population is predominantly engaged in rice cultivation, which involves prolonged contact with standing water and soil, both of which can harbour infective parasite stages. The absence of protective footwear, lack of handwashing facilities in the fields, and the use of untreated water for washing after work likely contribute to the high burden observed (10).

The strong association between low socioeconomic status and IPI positivity (aOR = 8.90; 97.1% of infected participants had low SES) highlights the social determinants of parasitic diseases. In Ethiopia, children from low-income households had a 6.66-fold higher risk of IPI (aOR=6.66; 95% CI: 1.87-23.7) (17). In Nepal, rural areas with economic marginalisation had significantly higher IPI prevalence (52.3%) compared to urban areas (32.4%) (19,20). The mechanisms linking poverty to IPIs are multiple; precarious housing with dirt floors facilitates contact with contaminated soil, lack of latrines leads to open defecation and environmental faecal contamination, limited education reduces awareness of hygiene practices; and low income restricts access to clean water, soap, and regular deworming (20).

Our results demonstrate that simple, low-cost interventions can be highly effective. Recent deworming (within 12 months) reduced the odds of IPI by 78%, and systematic deworming every six months reduced odds by 75%. This is consistent with WHO recommendations for preventive chemotherapy in endemic areas and with studies from Kenya showing that school-based MDA reduced IPI prevalence by 26.5% (21,22), and from Bangladesh showing that deworming combined with hygiene interventions significantly reduces geohelminth infections (23). The use of clean water and soap was independently associated with lower odds of IPI (aOR=0.25), which aligns with a meta-analysis showing that handwashing with soap reduces diarrhoeal disease risk by 30% in low-and-middle-income countries (24). The strong protective effect of safe food handling (aOR=0.05), defined as proper cooking, storage, and protection of food, is biologically plausible; cooking at ≥ 60°C inactivates most food-borne parasites, and proper storage prevents contamination by insect vectors or faecal matter (25).

Although diarrhoea or dysentery was reported by the majority of infected participants (91.4%) and showed a strong bivariate association with IPI, this association did not remain statistically significant after multivariate adjustment (p=0.06). This suggests that while these symptoms are good clinical indicators of possible IPI, they are not independent predictors when other risk factors (socioeconomic status, occupation, environmental exposure) are accounted for. Nevertheless, in resource-limited settings where diagnostic testing may not be available, the presence of persistent diarrhoea or dysentery should prompt empirical treatment or referral for stool examination.

The low proportion of infected participants who had consulted a health professional for their symptoms (22.9%) and the even lower proportion who had undergone prior stool examination (14.3%) highlight a major gap in case detection. This may reflect limited access to healthcare, low awareness of parasitic diseases, or a tendency toward self-medication with over-the-counter antiparasitic drugs, a practice that may contribute to emerging drug resistance (26).

This study has some limitations. First, the sample size is small (n=101), which limits

statistical power. A post-hoc calculation assuming 50% prevalence with 5% precision would have required approximately 384 participants. Second, the hospital-based design and a participation rate of 47% may introduce selection bias; findings may not be generalisable to the general adult population. Third, we examined only one stool sample per participant without concentration techniques (e. g. use of formoether). Therefore, the true prevalence is likely underestimated, particularly for protozoa and low-intensity helminth infections. Fourth, we could not distinguish *E. histolytica* from *E. dispar* by microscopy. Fifth, self-reported behavioral data are subject to recall and social desirability bias. Sixth, the multivariate model used stepwise selection in a small sample (n= 101, only 35 events), which may inflate type I error and yield unstable estimates. Consequently, the adjusted odds ratios should be interpreted as exploratory, not confirmatory. Finally, no formal sample size calculation was performed a priori. This study should therefore be considered hypothesis-generating. Confirmatory community-based studies using multiple stool samples and concentration techniques (or molecular methods) are urgently needed.

Despite these limitations, our findings suggest that extending deworming programmes to adults, particularly farmers in impoverished rural areas, and promoting safe food handling could reduce IPI burden. Future research should use multiple stool samples, concentration methods, and community-based sampling.

Conclusion:

In this hospital-based study of adults in rural Madagascar, the prevalence of intestinal parasitic infections was 34.7% using single sample direct microscopy. Low socioeconomic status, farming occupation, and living near untreated water sources were independently associated with higher odds of infection, while self-reported deworming within the preceding 12 months and safe food handling were associated with lower odds. Because of the small sample size, hospital-based design, participation bias, and low-sensitivity of the diagnostic method, these findings should be considered hypothesis-generating rather than definitive.

Nevertheless, our findings suggest that current control programmes, which mainly focus on school-aged children, may miss a substantial reservoir of infection among adults. Larger, community-based studies using multiple stool samples and concentration techniques (or molecular methods) are urgently needed to reliably estimate the true prevalence and to design integrated interventions that include adults, particularly farmers, in impoverished rural areas.

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Contributions of authors:

NJZ and AAR conceived and designed the study; LMR performed data collection; NJZ and LMR performed statistical analysis; and NJZ wrote the first draft of the manuscript. All authors read, revised, and approved the final manuscript.

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Original Article **Open Access**

Phenotypic and *ace-1*–mediated insecticide resistance in *Aedes* mosquitoes in Enugu North Senatorial Zone, Nigeria

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Abstract:

Background: Aedes mosquitoes are major vectors of important viral diseases such as Dengue Fever, Yellow Fever, Chikungunya, and Zika Virus Disease, which continue to pose serious public health challenges in many tropical regions largely due to the increasing resistance of mosquito vectors to commonly used insecticides. This study aimed to determine the insecticidal resistance status of *Aedes* mosquitoes in four surveyed communities within the Enugu North Senatorial Zone.
Methodology: Ovitrapping and larval sampling techniques were used to collect *Aedes* species from the selected communities (Orba, Umuopu, Obinagu, Neke, and Ekwegbe-Ulo). Fifty ovitraps were sited in each community to trap *Aedes* eggs. The traps were collected after 72 hours, and ribbons containing eggs were air-dried at room temperature and stored until needed. Larvae from hatched eggs were reared into adults. Immature stages of the mosquitoes were also collected from the field. Molecular identification of the mosquitoes was done using conventional PCR techniques. The mosquitoes were tested for susceptibility using the insecticides bendiocarb, deltamethrin, DDT, and pirimiphos-methyl. Data were analyzed using SPSS 21.0. One-way analysis (ANOVA) and Duncan Multiple Range Test (DMRT) were used to determine significant differences in the mean distribution and resistance of mosquitoes to insecticides in the selected communities, with $p<0.05$ considered statistically significant.
Results: The highest susceptibility of the mosquitoes across Orba, Umuopu, Obinagu, Neke, and Ekwegbe-Ulo was recorded for bendiocarb at 98%, 98%, 75%, 95%, and 100%, respectively, while the least was pirimiphos-methyl at 85%, 46%, 25%, 25%, and 100%, respectively. The highest knockdown efficacy was recorded with deltamethrin ($p<0.05$) compared to other insecticides. Comparably, no significant ($p>0.05$) difference in the exposed adults with DDT compared to other insecticides. The molecular detection assay implicated the *ace-1* resistance gene as one of the major genes responsible for resistance to insecticides in mosquitoes in the area.
Conclusion: *Aedes* mosquitoes from the selected communities showed different responses to the insecticides tested. Bendiocarb was the most effective, while pirimiphos-methyl was the least effective. The detection of the *ace-1* gene indicates the presence of insecticide resistance among the mosquito populations. Regular monitoring of insecticide resistance and proper use of insecticides should be encouraged. Integrated mosquito control measures should also be strengthened to improve vector control effectiveness.

Keywords: Phenotypic; Insecticide resistance; *Aedes* mosquitoes; Enugu North Senatorial Zone; Nigeria

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Résistance phénotypique et résistance aux insecticides médiée par *ace-1* chez les moustiques *Aedes* dans la zone sénatoriale nord d'Enugu, au Nigéria

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Résumé:

Contexte: Les moustiques du genre Aedes sont des vecteurs majeurs de maladies virales importantes telles que la dengue, la fièvre jaune, le chikungunya et le virus Zika, qui continuent de poser de sérieux problèmes de santé publique dans de nombreuses régions tropicales, principalement en raison de la résistance croissante des moustiques vecteurs aux insecticides couramment utilisés. Cette étude visait à déterminer le niveau de résistance aux insecticides des moustiques du genre Aedes dans quatre communautés étudiées au sein de la zone sénatoriale d'Enugu Nord.
Méthodologie: Des techniques de piégeage des œufs et d'échantillonnage larvaire ont été utilisées pour collecter des moustiques du genre Aedes dans les communautés sélectionnées (Orba, Umuopu, Obinagu, Neke et Ekwegbe-Ulo). Cinquante pièges à œufs ont été installés dans chaque communauté. Les pièges ont été relevés après 72 heures, et les rubans contenant les œufs ont été séchés à l'air libre à température ambiante puis conservés jusqu'à leur utilisation. Les larves issues des œufs éclos ont été élevées jusqu'au stade adulte. Les stades immatures des moustiques ont également été collectés sur le terrain. L'identification moléculaire des moustiques a été réalisée par PCR conventionnelle. La sensibilité des moustiques a été testée aux insecticides bendiocarb, deltaméthrine, DDT et pirimiphos-méthyl. Les données ont été analysées à l'aide du logiciel SPSS 21.0. Une analyse de variance (ANOVA) et le test de Duncan ont été utilisés pour déterminer les différences significatives dans la distribution moyenne et la résistance des moustiques aux insecticides au sein des communautés sélectionnées, avec un seuil de signification statistique fixé à $p<0,05$.
Résultats: La plus grande sensibilité des moustiques à Orba, Umuopu, Obinagu, Neke et Ekwegbe-Ulo a été observée pour le bendiocarbe (98%, 98%, 75%, 95% et 100%, respectivement), tandis que la plus faible a été enregistrée pour le pirimiphos-méthyl (85%, 46%, 25%, 25% et 100%, respectivement). L'efficacité paralysante la plus élevée a été observée avec la deltaméthrine ($p<0,05$) par rapport aux autres insecticides. En revanche, aucune différence significative ($p>0,05$) n'a été constatée chez les adultes exposés au DDT par rapport aux autres insecticides. L'analyse moléculaire a révélé que le gène de résistance *ace-1* est l'un des principaux gènes responsables de la résistance aux insecticides chez les moustiques de la région.
Conclusion: Les moustiques Aedes des communautés sélectionnées ont présenté des réponses différentes aux insecticides testés. Le bendiocarbe s'est avéré le plus efficace, tandis que le pirimiphos-méthyle était le moins efficace. La détection du gène *ace-1* indique la présence d'une résistance aux insecticides au sein des populations de moustiques. Un suivi régulier de la résistance aux insecticides et une utilisation appropriée de ces derniers doivent être encouragés. Les mesures intégrées de lutte antivectorielle doivent également être renforcées afin d'améliorer l'efficacité de la lutte antivectorielle.

Mots-clés: Phénotypique; Résistance aux insecticides; Moustiques *Aedes*; Zone sénatoriale d'Enugu Nord; Nigéria

Introduction:

Insecticide resistance among mosquito vectors has become a major challenge to vector control and public health programmes worldwide (1). *Aedes aegypti* and *Aedes albopictus* are important vectors of dengue fever, yellow fever, chikungunya, and zika virus disease (2). Although pyrethroids and organophosphates are widely used for mosquito control because of their rapid action (1), their prolonged and indiscriminate use in agriculture, public health, and domestic settings has contributed to the emergence of resistant mosquito populations (3).

Phenotypic resistance occurs when mosquitoes survive insecticide doses that would normally kill susceptible populations (1). Recent studies have shown increasing resistance of *Aedes* mosquitoes to pyrethroids, carbamates, organophosphates, and organochlorines in many parts of the world (4,5). This growing resistance threatens the effectiveness of vector control programmes and may increase the risk of arboviral disease transmission (1).

Resistance in mosquitoes is associated with both target-site and metabolic mechanisms (3). One important mechanism involves mutations in the Ace-1 gene, which reduce the sensitivity of mosquitoes' acetylcholinesterase-1 enzyme (AChE) to organophosphate and carbamate insecticides (1,6). Increased activities of detoxification enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases, and esterases also contribute to insecticide resistance and cross-resistance (7).

Environmental and human-related fact-

ors such as urbanization, poor sanitation, indiscriminate insecticide use, climate change, and increased human movement further promote the spread of resistant mosquito populations (1, 8). In Nigeria, most resistance studies have focused on *Anopheles* mosquitoes, while limited information exists on *Aedes* mosquitoes, especially in southeastern Nigeria. Previous Nigerian studies have reported pyrethroid resistance and knockdown resistance mutations in *Aedes* populations (9,10). Therefore, this study investigated the phenotypic resistance status and *ace-1*-mediated resistance mechanisms in *Aedes* mosquitoes collected from the Enugu North Senatorial Zone, Nigeria.

Materials and method:

Study area:

This study was conducted in Enugu North Senatorial Zone (Fig 1). The Local Government Areas (LGAs) located in this senatorial zone falls within the tropical rain forest zone of Nigeria. Typically, it has two seasons; rainy season (from late March to late October) and dry season (from November to early March). As at 2016 censors, Enugu senatorial zone has a population of 1,490,100. The climate of the senatorial zone is tropical with a mean monthly temperature fluctuating between 26.7^{o}C and 29^{o}C.

Also, the senatorial zone (Nsukka region) houses the first Nigerian autonomous University, University of Nigeria, Nsukka (UNN), which was founded by Dr. Nnamdi Azikiwe the first president of Nigeria. However, the people of the senatorial zone are majorly farmer. Presently, the senatorial zone comprises people from different occupational backgrounds ranging from academics, civil servants, farmers, craftsmen, business men and women.

Study site selection and ethical approval:

Five communities were selected from five local government areas, within Enugu North Senatorial Zone for fair representation. The communities to be visited are: Obinagu, Ekwegbe Ulo, Neke, Umuopu and Orba. Enugu north senatorial zone is a sub-urban town in South- East Nigeria in Enugu state, located on a plateau at an elevation of 429 meters above sea level (11). It is bordered to the south by plains of high land of the Udi area and to the north by Nkalagu and Okutu plains (12). The approval of the heads of the selected communities was obtained.

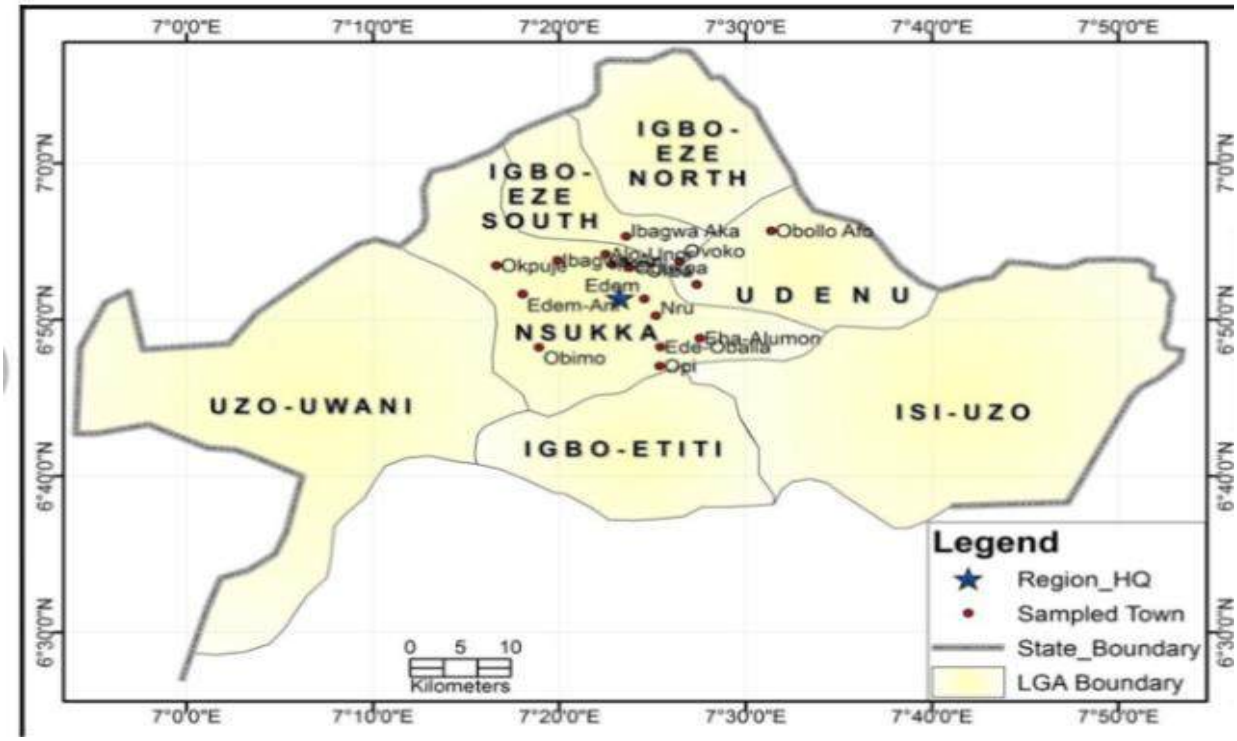


Fig. 1: Map of Enugu north senatorial zone (Source: Google.com)

Study design:

The study was an observational descriptive cross-sectional design. *Aedes* mosquitoes sampling was carried using varied entomological techniques which include ovitrap setting, larval survey and modified Human Landing Catch (mHLC). Samples collected were subjected to different entomological procedures designed to obtain descriptive data. Thus, adult *Aedes* mosquitoes collected by mHLC were identified by morphology and molecular assays, while ovitrap collected *Aedes* mosquito eggs were reared to adults before they were morphological identified, properly preserved and store for assays.

The assays included phenotypic susceptibility/resistance detection and molecular (whole genome sequencing, next generation sequencing, target site mutation and metabolic resistance) assays.

Study duration:

This study collected information once in a month on vector abundance and distribution, vector species composition, feeding and resting behaviour and nature of breeding sites. Thus, the three consecutive days sampling for data was carried out within a month in the rainy season of 2023. Assay took one month, bringing the duration of the project to two months.

Entomological surveillance:

The following entomological techniques were used to collect *Aedes* species from the selected communities:

Ovitrapping:

Ovitraps were designed to collect *Aedes* mosquitoes' eggs. Each ovitrap contained a cylindrical cup (about 200ml, containing about 200 ml water), lined with a white ribbon on which the mosquitoes laid eggs just above the water level. Fifty ovitraps were sited in each community and each were placed on the ground within vegetations in and around houses. The traps were collected after 72 hours (three days) and ribbons containing eggs were air-dried under room temperature and properly stored or soaked. Larvae from hatched eggs were then be reared to adults.

Larval sampling:

This method was used to collect immature stages of domestic and peri-domestic *Aedes* mosquitoes (e.g. *A. aegypti* and *A. albopictus*) for three days. Natural habitats such as tree hole and leaf axils, which might harbour immature stages (larvae and/or pupae) of *Aedes* species, were inspected. Likewise, artificial containers in and around the houses were inspected for the presence of larvae and pupae of the vectors.

In order to make the sampling representative, 50 houses were randomly sampled in each community (13). Where possible, the entire water content of a container was emptied into a bowl and the immature stages picked using disposable pipettes. They were introduced into well-labelled containers, taken to a mini-insectary and reared to adults. Larval sampling showed the distribution of the vectors and made vector sampling representative. It also captured the diversity of *Aedes* species in an area since both natural and artificial containers breeders were sampled.

Mosquito identification and preservation:

Adult mosquitoes collected in the field and those reared to adults from immature (eggs and larvae) collections were immobilized using ethyl acetate. The specimens were morphologically identified to species level using appropriate taxonomic keys. They were introduced into well-labelled Eppendorf tubes containing RNA-later and stored at 20°C for further analysis.

WHO tube bio-assay for vector insecticide susceptibility/resistance:

This assay was carried out in accordance with WHO tube bio-assay protocol (13). Mosquitoes from each community were pooled (by soaking egg-containing ribbons from the community together) and healthy, female, non-blood-fed, 3-5 days old *Aedes* mosquitoes were used. There were 6 holding or pre-exposure tubes (4 replicate and 2 control tubes). About 20-25 mosquitoes were aspirated into each tube and held for one hour after which, moribund (unhealthy) mosquitoes were removed and replaced with healthy ones. Thereafter, the mosquitoes were transferred into the corresponding exposure tubes (4 tubes containing the insecticides impregnated papers and 2 control tubes).

Knockdown were recorded at the 10^{th}, 15^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th} minutes. Then the mosquitoes were transferred to the holding of post-exposure tubes and provided with 10% sugar-soaked balls of cotton wool. Mortalities were recorded at the 24^{th} hour. From 98-100% indicates that the mosquitoes are susceptible to the insecticide used. Mortality of about 90-97% indicates suspected that will need to be confirmed by a repeat of the process, and mortality below 90% indicates resistance.

Four insecticides were tested on *Aedes* mosquitoes collected from each community; Bendiocarb, Deltamethrin, DDT and Pirimiphos-methyl. These insecticides represent carbamates, pyrethroids, organochlorine and organophosphate insecticide classes respectively.

Insecticide resistant mosquitoes were immobilized with chloroform and preserved using dessicant poured into micro tubes which were properly labelled with dates, numbers, species, sex and resistance status of the mosquitoes. All the micro tubes containing preserved insecticide resistant mosquitoes were put in micro tube boxes and stored at -20^{o}C in the National Arbovirus Research Centre (NAVRC), Enugu State, for future use.

Molecular insecticidal resistance assay:

Aedes mosquito samples stored in the dessicant at -20^{o}C were retrieved, prepared and subjected to PCR assay to detect the resistant gene (*ace-1*) as the principal resistance in the population, in accordance with the protocol described by Oguzie et al., (14).

DNA extraction and PCR assay:

This was done using Zymo Research DNA kit extraction from USA commercially purchased from Inqaba Biotec West Africa. The PCR master mix (reaction setup) contained One Taq 2x Master Mix (14µl), 2µl EX2AGDIR 1 (forward primer) (AGGTCACGGTGAGTCCGTA CGA), 2 µl EX4AGREV 2 (reverse primer) (AGGGCGGACAG CAGATGCAGCGA), nuclease free water (9 µl), DNA template (3 µl), to make a total PCR reaction volume of 30 µl.

The PCR amplification protocol was adopted and modified from the published study by Fanello et al., (15) and consisted of initial denaturation at 95^{o}C for 90 secs, followed by 35 cycles of denaturation at 94^{o}C for 30 secs, annealing at 68^{o}C for 30secs and extension at 72^{o}C for 60 secs, and a final extension at 72^{o}C for 5 mins.

Gel electrophoresis:

2% agarose gel was prepared and used for electrophoresis of the PCR amplicons at 120 volts for a period of 1 hour 30 mins (16). The expected band size of the amplicon was 924 bp

Data analysis:

Data were entered using Microsoft Excel and analyzed using the Statistical Packages for Social Sciences (SPSS version 21.0). All graphs were generated with Microsoft Excel. The numbers of dead and surviving mosquitoes were recorded after 24 hours. The percentages for mortality and survival were determined by dividing the number of dead or surviving mosquitoes in each insecticide treatment by the total number of mosquitoes exposed and multiplying by 100.

Abbott's formula was applied when needed to correct for control mortality as per WHO guidelines. The mean mortality efficacy was calculated by summing the number of dead mosquitoes recorded in all the four replicates and dividing by the number of replicates. Results are presented as mean ± standard error (SE). One-way analysis (ANOVA) and Duncan Multiple Range Test (DMRT) were used to determine significant differences in the mean distribution and resistance of mosquito species in the selected communities at $p<0.05$ (95% confidence interval).

Results:

Susceptibility of adult mosquitoes to the different insecticides:

The percentage (%) susceptibility of the adult mosquitoes to the insecticides according to locations is presented in Fig 2. The insecticide with the highest percentage susceptibility across the study sites at Orba, Umuopu, Obinagu, Neke, and Ekwegbe-Ulo was bendiocarb with 98%, 98%, 75%, 95% and 100% respectively, while the least were pirimiphos-methyl with 85%, 46%, 25%, 25% and 100% respectively.

The percentage (%) susceptibility according to study sites in decreasing order were as follows: deltamethrin: Orba and Ekwegbe Ulo >Umuopu>Obinagu>Neke; bendiocarb: Ekwegbe Ulo>Orba and Umuopu>Neke>Obinagu; pirimiphos-methyl: Ekwegbe Ulo>Orba>Umuopu>Obinagu and Neke; and DDT: Neke>Umuopu>Ekwegbe Ulo>Orba and Obinagu.

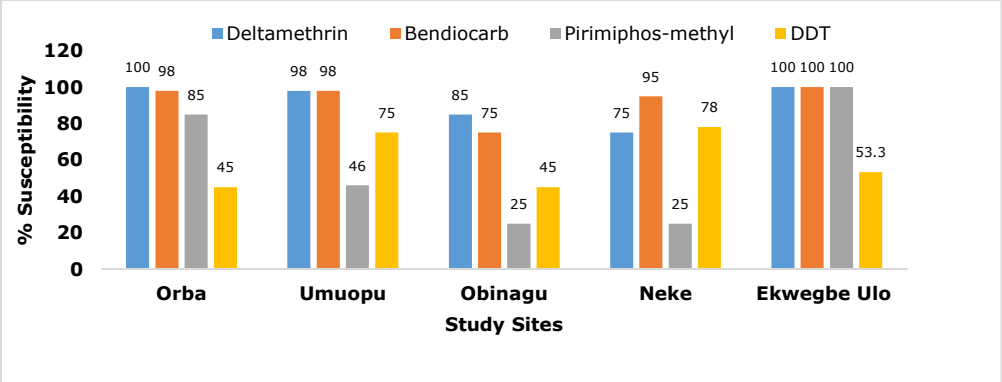


Fig 2: Percentage susceptibility of adult mosquitoes to the insecticides according to location

The rate and time of knockdown (% knockdown time) of adult mosquitoes exposed to different insecticides according to locations and duration are presented in Figs 3-6. It was observed that only deltamethrin achieved above 50% knockdown time (KDT) in the adult mosquitoes at 10 minutes' exposure across all the studied sites. This was followed by bendiocarb and DDT insecticides which knocked down more than half (> 50%) of the adult mosquitoes when exposed for 30 minutes for all study sites. Bendiocarb exposure in Neke community achieved 39.3% knockdown rate. The knockdown rate of adult mosquitoes to pirimiphos-methyl was the lowest (< 15%), with few mosquitoes knocked down even at 1-hour exposure across the study sites.

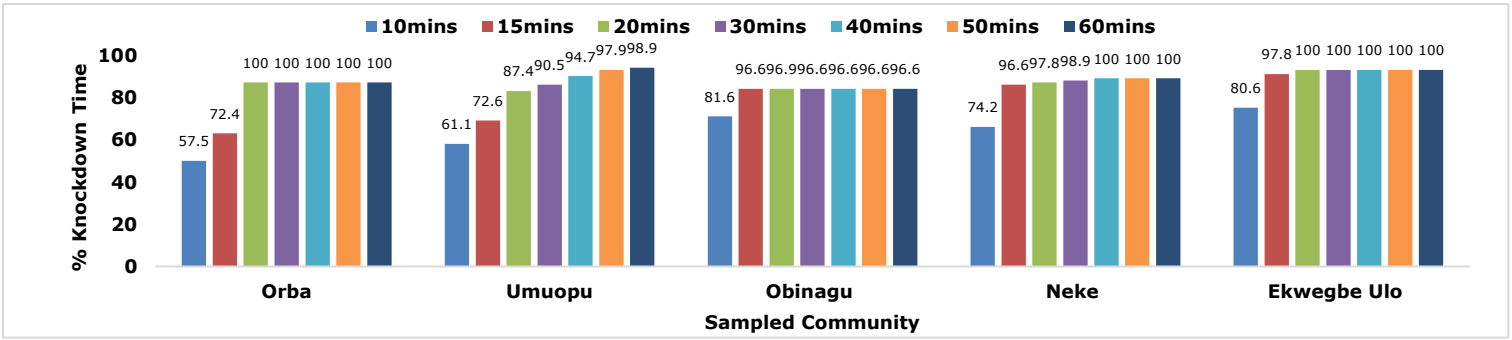


Fig 3: Percentage (%) knockdown time (KDT) of *Aedes* mosquito by deltamethrin across the communities

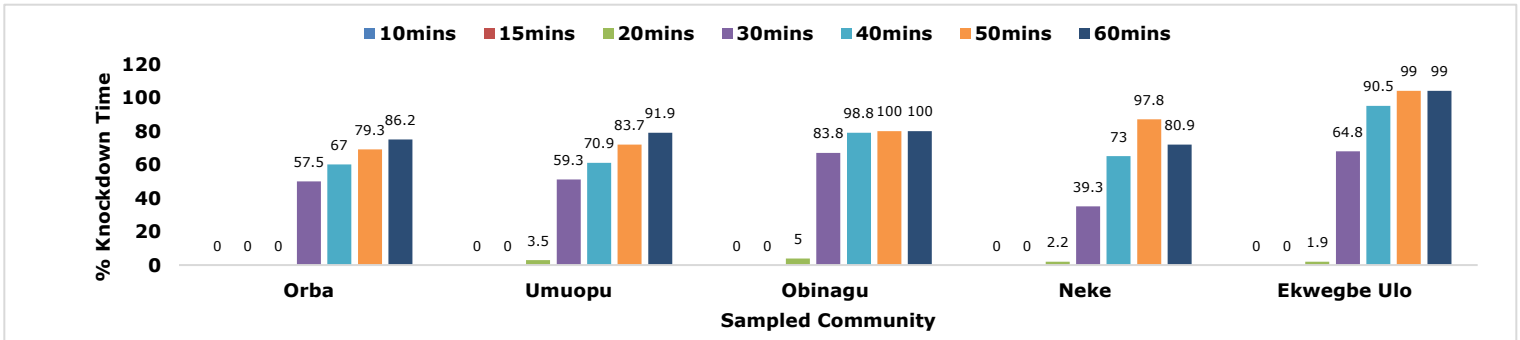


Fig 4: Percentage (%) knockdown time (KDT) of *Aedes* mosquito by bendiocarb across the studied communities

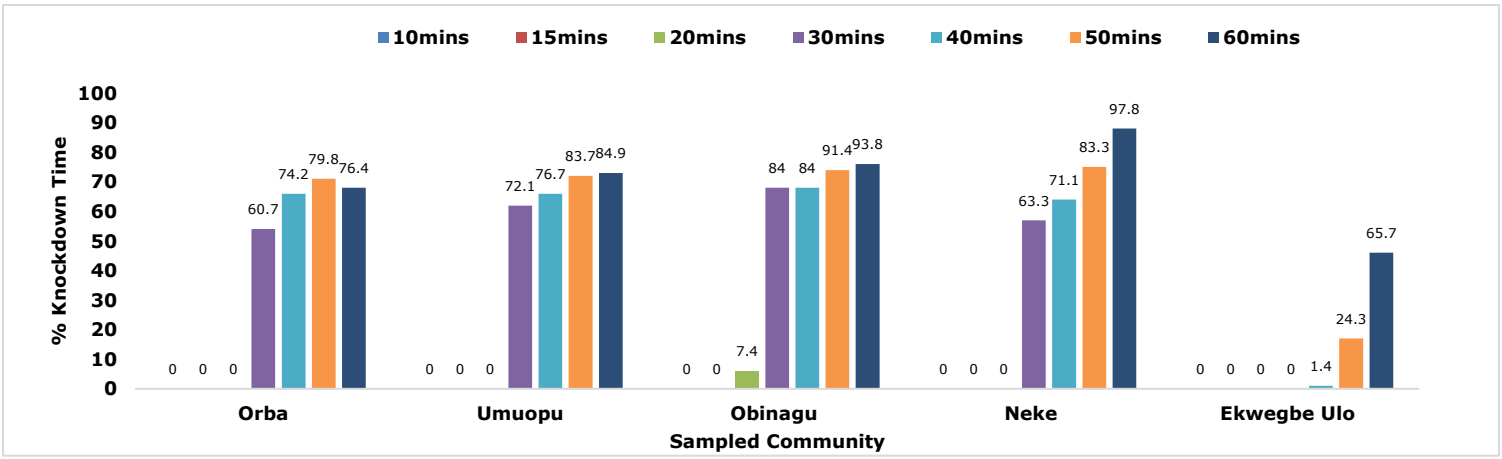


Fig 5: Percentage (%) knockdown time of (KDT) *Aedes* mosquito by pirimiphos-methyl across the studied communities

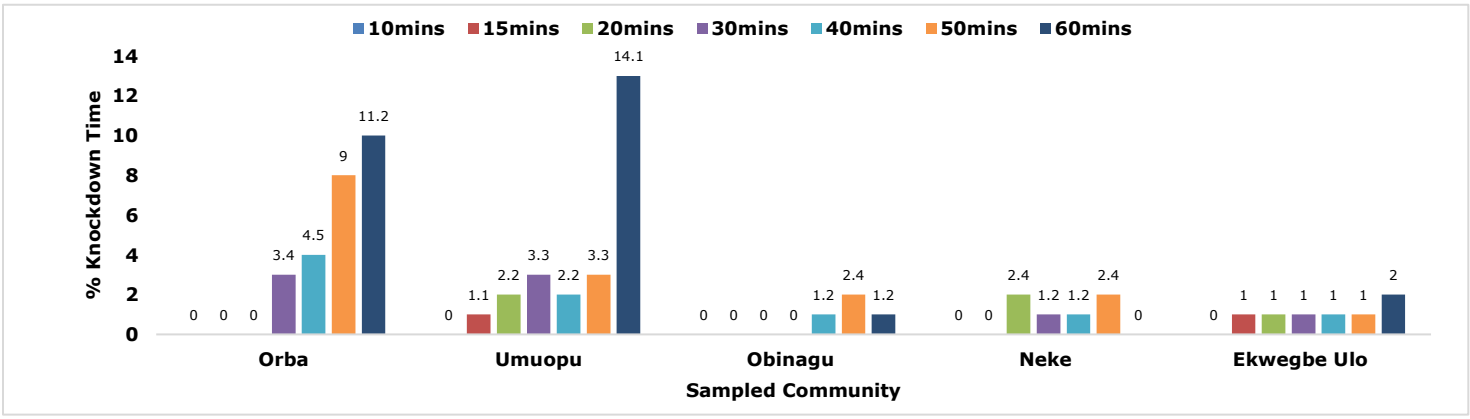


Fig 6: Percentage (%) knockdown time (KDT) of *Aedes* mosquito by DDT across the studied communities

Table 2 shows the knockdown efficacy of different insecticides on adult mosquitoes according to duration and locations. When the knockdown efficacy of the insecticides was compared for each study site according to exposure duration, deltamethrin efficacy for Orba community was significantly (p<0.05) higher within 10, 15 and 20 mins of exposure, than what was observed from 30 mins and above. Conversely, the knockdown efficacy of bendiocarb, pirimiphos-methyl and DDT only significantly (p< 0.05) increased within 40 to 60 mins of exposure of adult mosquitoes to it.

At Umuopu community, deltamethrin knockdown efficacy varied significantly (p<0.05) with time, with 40 to 60 mins exposure higher in efficacy than what was recorded at 10 and 15 mins. Obinagu, Neke and Ekwegbe Ulo communities recorded non-significant (p>0.05) differences in both deltamethrin and pirimiphos-methyl knockdown efficacy with respect to exposure duration, whereas bendiocarb and DDT had significantly (p<0.05) higher efficacy from 30 to 60 mins.

When the different insecticides were compared, it was observed that at Orba community, deltamethrin had significantly (p<0.05) higher efficacy than the rest at 10 to 20 mins exposure, while bendiocarb and DDT became higher (p<0.05) with prolonged exposure of 30 to 60 mins. However, other communities showed that deltamethrin had overall higher (p< 0.05) knockdown efficacy than the rest of the insecticides irrespective of duration exposure, except in Ekwegbe Ulo community which showed a comparable knockdown efficacy of bendiocarb to that of deltamethrin (p>0.05) at 40 to 60 mins of expos ure.

However, when the efficacy of each insecticide was compared across study sites, deltamethrin had a lower (p<0.05) efficacy in Orba than others. The knockdown efficacy of bendiocarb was higher (p<0.05) in Obinagu and Ekwegbe Ulo at 30 to 60 mins, while susceptibility to DDT was higher in Obinagu at 20 mins exposure. Pirimiphos-methyl efficacy was significantly (p<0.05) higher at 60 mins in Orba and Umuopu than other communities.

Table 2: Knockdown efficacy of different insecticides on adult mosquitoes according to duration and locations

Study site	Duration	Insecticide			
		Deltamethrin	**Bendiocarb**	**Pirimiphos-methyl**	**DDT**
Orba	10 mins	12.50 ± 0.65^{b2A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	15 mins	15.75 ± 0.85^{b2A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	20 mins	21.75 ± 0.85^{b2A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	30 mins	0.00 ± 0.00^{a1A}	12.50 ± 1.32^{ab2AB}	0.75 ± 0.25^{ab1A}	13.50 ± 0.65^{ab2A}
	40 mins	0.00 ± 0.00^{a1A}	15.00 ± 0.91^{b2A}	1.00 ± 0.41^{ab1A}	16.50 ± 0.29^{b2A}
	50 mins	0.00 ± 0.00^{a1A}	17.25 ± 0.48^{b23A}	2.00 ± 0.41^{b2A}	17.75 ± 0.63^{b3A}
	60 mins	0.00 ± 0.00^{a1A}	18.75 ± 0.25^{b3A}	2.50 ± 0.29^{b2B}	17.00 ± 0.41^{b23A}
Umuopu	10 mins	14.50 ± 1.04^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	15 mins	17.25 ± 0.48^{a2A}	0.00 ± 0.00^{a1A}	0.25 ± 0.25^{a1A}	0.00 ± 0.00^{a1A}
	20 mins	20.75 ± 0.48^{ab2A}	0.75 ± 0.48^{ab1A}	0.50 ± 0.29^{a1A}	0.00 ± 0.00^{a1A}
	30 mins	21.50 ± 0.50^{ab3B}	12.75 ± 0.95^{bc2AB}	0.75 ± 0.25^{a1A}	15.50 ± 0.65^{b23A}
	40 mins	22.50 ± 0.50^{b3AB}	15.25 ± 0.48^{bc2A}	0.50 ± 0.29^{a12A}	16.50 ± 0.29^{b23A}
	50 mins	23.25 ± 0.48^{b2B}	18.00 ± 0.41^{c12A}	0.75 ± 0.25^{a1A}	18.00 ± 0.41^{b12A}
	60 mins	23.50 ± 0.50^{b3B}	19.75 ± 0.25^{c23AB}	3.25 ± 0.48^{b12B}	18.25 ± 0.48^{b2A}
Obinagu	10 mins	17.75 ± 1.25^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	15 mins	21.00 ± 0.41^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	20 mins	21.00 ± 0.41^{a2A}	1.00 ± 0.41^{a1A}	0.00 ± 0.00^{a1A}	1.50 ± 0.65^{a12B}
	30 mins	21.00 ± 0.41^{a2B}	16.75 ± 0.75^{b12B}	0.00 ± 0.00^{a1A}	17.00 ± 0.82^{b12A}
	40 mins	21.00 ± 0.41^{a3AB}	19.75 ± 0.25^{b23B}	0.25 ± 0.25^{a1A}	17.00 ± 0.91^{b2A}
	50 mins	21.00 ± 0.41^{a2AB}	20.00 ± 0.00^{b2AB}	0.50 ± 0.29^{a1A}	18.50 ± 0.96^{b2A}
	60 mins	21.00 ± 0.41^{a2AB}	20.00 ± 0.00^{b2B}	0.25 ± 0.25^{a1A}	19.00 ± 0.71^{b2AB}
Neke	10 mins	16.50 ± 0.65^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	15 mins	21.50 ± 1.19^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	20 mins	21.75 ± 1.11^{a2A}	0.50 ± 0.50^{a1A}	0.50 ± 0.29^{a1A}	0.00 ± 0.00^{a1A}
	30 mins	22.00 ± 1.22^{a3B}	8.75 ± 0.85^{b2A}	0.25 ± 0.25^{a1A}	14.25 ± 1.93^{b23A}
	40 mins	22.25 ± 1.03^{a2B}	16.25 ± 1.03^{c12A}	0.25 ± 0.25^{a1A}	16.00 ± 2.16^{b12A}
	50 mins	22.25 ± 1.03^{a2B}	21.75 ± 0.48^{c2B}	0.50 ± 0.29^{a1A}	18.75 ± 0.85^{b12A}
	60 mins	22.25 ± 0.85^{a2B}	18.00 ± 0.71^{c12A}	0.00 ± 0.00^{a1A}	22.00 ± 0.41^{c2B}
Ulo Ekwegbe	10 mins	18.75 ± 0.65^{a2B}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
	15 mins	22.75 ± 0.50^{a2B}	0.00 ± 0.00^{a1A}	0.25 ± 0.25^{a1A}	0.00 ± 0.00^{a1A}
	20 mins	23.25 ± 0.48^{a2B}	0.50 ± 0.50^{a1A}	0.25 ± 0.25^{a1A}	0.00 ± 0.00^{a1A}
	30 mins	23.25 ± 0.48^{a2B}	17.00 ± 1.03^{b2B}	0.25 ± 0.25^{a1A}	0.00 ± 0.00^{a1A}
	40 mins	23.25 ± 0.48^{a2B}	23.75 ± 1.11^{b2B}	0.25 ± 0.25^{a1A}	0.33 ± 0.03^{a1A}
	50 mins	23.25 ± 0.48^{a3B}	26.00 ± 0.85^{b3B}	0.25 ± 0.25^{a1A}	5.67 ± 3.85^{ab2A}
	60 mins	23.25 ± 0.48^{a3B}	26.00 ± 0.85^{b3B}	0.50 ± 0.29^{a1A}	15.33 ± 2.16^{b2A}

[1]All values expressed as mean ± standard error (±SE).
[2]Different superscript small letters indicate significant difference ($p < 0.05$) in knockdown efficacy mean values within duration for each study site (columns) using Kruskal-Wallis' test.
[3]Different superscript numerals indicate significant difference ($p < 0.05$) in knockdown efficacy mean values among insecticides for each study site (rows) using Kruskal-Wallis' test.
[4]Different superscript capital letters indicate significant difference ($p < 0.05$) in knockdown efficacy mean among study sites for each duration (columns) using Kruskal-Wallis' test.

Mortality of adult mosquitoes to the insecticides:

The overall mortality rate of adult mosquitoes exposed to the different insecticides according to locations is presented in Table 3. It was observed that adult mosquitoes exposed to DDT and pirimiphos-methyl had high mortality rates (>50%) across the study sites, except at Neke community where mortality rate of pirimiphos-methyl-exposed mosquitoes was 14.3%. At Ekwegbe Ulo community, all the insecticides showed 100% mortality efficacy on the mosquitoes, except for DDT with 57.1% mortality.

The survival rate of exposed adult mosquitoes after 24 hours recovering of insecticides knockdown was high for deltamethrin (89.7%) and bendiocarb (88.5%) in Orba. The same trend survival rate at above 50% was recorded for adult mosquitoes exposed to deltamethrin and bendiocarb across the other study sites.

Also, adult mosquitoes exposed to pirimiphos-methyl had high percentage survival (85.7% at Neke. There was no survival of the mosquitoes exposed to deltamethrin, bendiocarb and pirimiphos-methyl (0%) in Ekwegbe Ulo community, but those exposed to DDT had 42.9% survival. Hence, the test populations of *Aedes* mosquitoes across the studied communities were largely resistant to the insecticides, except in Ekwegbe Ulo community that recorded mosquitoes 100% mortality to deltamethrin, bendiocarb and pirimiphos-methyl.

Table 3: Overall mortality rate of adult mosquitoes exposed to different insecticides in the study area

Study Site	Insecticide	% Mortality		% Survival	
		Test Group	Control Group	Test Group	Control Group
Orba	Deltamethrin	9 (10.3)	40 (97.6)	78 (89.7)	1 (2.4)
	Bendiocarb	10 (11.5)	46 (100.0)	77 (88.5)	0 (0.0)
	Pirimiphos-methyl	71 (79.8)	45 (100.0)	18 (20.2)	0 (0.0)
	DDT	62 (69.7)	46 (100.0)	27 (30.3)	0 (0.0)
Umuopu	Deltamethrin	14 (14.7)	46 (100.0)	81 (85.3)	0 (0.0)
	Bendiocarb	10 (11.6)	43 (100.0)	76 (88.4)	0 (0.0)
	Pirimiphos-methyl	69 (75.0)	40 (100.0)	23 (25.0)	0 (0.0)
	DDT	58 (67.4)	0 (0.0)	28 (32.6)	0 (0.0)
Obinagu	Deltamethrin	12 (13.8)	43 (95.6)	75 (86.2)	2 (4.4)
	Bendiocarb	10 (12.5)	0 (0.0)	70 (87.5)	0 (0.0)
	Pirimiphos-methyl	70 (82.4)	40 (97.6)	15 (17.6)	1 (2.4)
	DDT	61 (75.3)	40 (100.0)	20 (24.7)	0 (0.0)
Neke	Deltamethrin	13 (14.6)	40 (100.0)	76 (85.4)	0 (0.0)
	Bendiocarb	10 (11.2)	0 (0.0)	79 (88.8)	0 (0.0)
	Pirimiphos-methyl	12 (14.3)	40 (100.0)	72 (85.7)	0 (0.0)
	DDT	50 (55.6)	41 (100.0)	40 (44.4)	0 (0.0)
Ekwegbe Ulo	Deltamethrin	93 (100.0)	0 (0.0)	0 (0.0)	46 (100.0)
	Bendiocarb	105 (100.0)	0 (0.0)	0 (0.0)	46 (100.0)
	Pirimiphos-methyl	100 (100.0)	0 (0.0)	0 (0.0)	46 (100.0)
	DDT	40 (57.1)	0 (0.0)	30 (42.9)	46 (100.0)

[1]Frequency percentage in parenthesis (%).
[2][98% – 100% mortality: test population is susceptible; 90% – 97% mortality**:** test population has possible resistance; below 90% mortality: test population is resistant (WHO, 2016)].

Table 4 shows the mortality efficacy of different insecticides on adult mosquitoes according to treatment groups and locations. When the mean mortality efficacy of the insecticides was compared for each study site according to treatment groups, none of the tested insecticides significantly (p>0.05) varied with control group in all the study sites. When the different insecticides were compared, it was observed that at Orba and Obinagu communities, adult mosquitoes exposed to pirimiphos-methyl and DDT showed a significantly (p< 0.05) higher mortality efficacy than deltamethrin and bendiocarb. At Umuopu community, pirimiphos-methyl achieved significantly (p< 0.05) higher mortality efficacy among adult mosquitoes than the efficacy of deltamethrin and bendiocarb, but comparable with that achieved by DDT (p>0.05).

The mortality efficacy of DDT recorded at Neke community was significantly (p<0.05) higher than the rest of insecticides in the community. At Ekwegbe Ulo community, the mortality efficacy of deltamethrin, bendiocarb and pirimiphos-methyl were higher (p<0.05) than that of DDT. When the mortality efficacy of each insecticide was compared across study sites, DDT had no significant (p>0.05) difference on the exposed adult mosquitoes. Deltamethrin and bendiocarb mortality efficacy was higher (p< 0.05) in Ekwegbe Ulo than the other communities. It was observed that pirimiphos-methyl mortality efficacy recorded in Neke community was significantly (p < 0.05) lower than what was recorded for other communities.

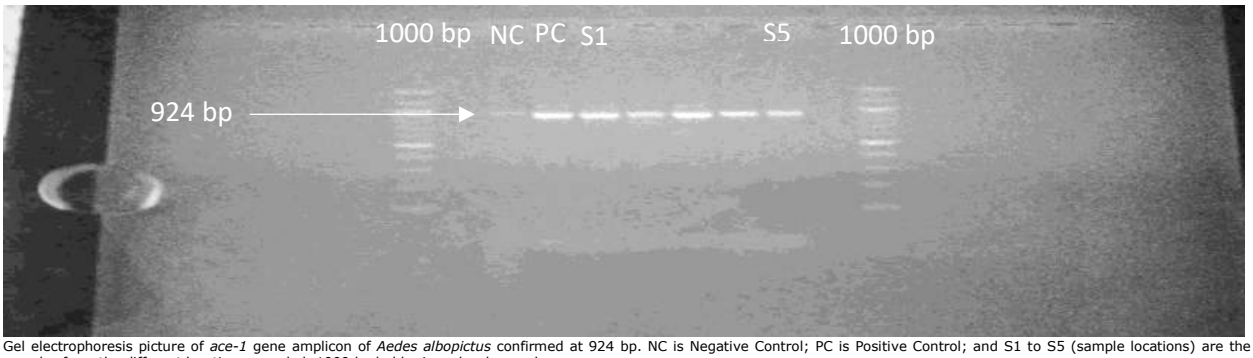
Table 4: Mortality efficacy of different insecticides on adult mosquitoes according to treatment and locations

Study Site	Treatment	Insecticide			
		Deltamethrin	**Bendiocarb**	**Pirimiphos-methyl**	**DDT**
Orba	Test group	2.25 ± 0.63^{a1A}	2.50 ± 0.65^{a1A}	17.75 ± 0.48^{a2B}	15.50 ± 0.65^{a2A}
	Control group	20.00 ± 0.00^{a1A}	23.00 ± 1.00^{a1A}	22.50 ± 0.50^{a1A}	23.00 ± 1.00^{a1A}
Umuopu	Test group	3.50 ± 0.65^{a12A}	2.50 ± 0.29^{a1A}	17.25 ± 0.75^{a3B}	14.50 ± 0.65^{a23A}
	Control group	23.00 ± 1.00^{a1A}	21.50 ± 0.50^{a1A}	20.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}
Obinagu	Test group	3.00 ± 1.08^{a1A}	2.50 ± 0.65^{a1A}	17.50 ± 0.65^{a2B}	15.25 ± 1.11^{a2A}
	Control group	21.50 ± 0.50^{a1A}	0.00 ± 0.00^{a1A}	20.00 ± 0.00^{a1A}	20.00 ± 0.00^{a1A}
Neke	Test group	3.25 ± 0.48^{a1A}	2.50 ± 0.65^{a1A}	3.00 ± 0.41^{a1A}	12.50 ± 0.65^{a2A}
	Control group	20.00 ± 0.00^{a1A}	0.00 ± 0.00^{a1A}	20.00 ± 0.00^{a1A}	20.50 ± 0.50^{a1A}
Ulo Ekwegbe	Test group	23.25 ± 0.65^{a2B}	26.25 ± 1.11^{a2B}	25.00 ± 1.08^{a2B}	13.33 ± 4.75^{a1A}
	Control group	23.00 ± 1.00^{a1A}	23.00 ± 1.00^{a1A}	23.00 ± 1.00^{a1A}	23.00 ± 1.00^{a1A}

[1]All values expressed as mean ± standard error (±SE).
[2]Different superscript small letters indicate significant difference ($p < 0.05$) in mortality efficacy mean values between treatments for each study site (columns) using Mann-Whitney test.
[3]Different superscript numerals indicate significant difference ($p < 0.05$) in mortality efficacy mean values among insecticides for each study site (rows) using Kruskal-Wallis test.
[4]Different superscript capital letters indicate significant difference ($p < 0.05$) in mortality efficacy mean among study sites for treatment group (columns) using Kruskal-Wallis test.

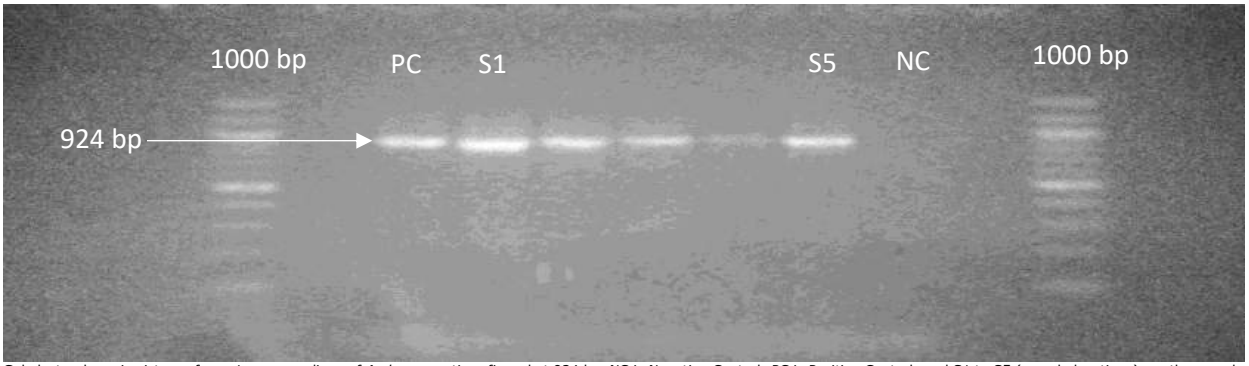
Insecticide resistance (*ace-1*) gene in the mosquitoes:

The gel electrophoresis pictures of the PCR amplicons showed that *ace-1* (resistance) gene was amplified at 924 bp band in *Aedes albopictus* (Fig 7) and *Aedes aegypti* (Fig 8).



Gel electrophoresis picture of *ace-1* gene amplicon of *Aedes albopictus* confirmed at 924 bp. NC is Negative Control; PC is Positive Control; and S1 to S5 (sample locations) are the samples from the different locations sampled, 1000 bp ladder is molecular marker

Fig 7: Gel electrophoresis picture of *ace-1* amplicon of *Aedes albopictus*



Gel electrophoresis picture of *ace-1* gene amplicon of *Aedes aegypti* confirmed at 924 bp. NC is Negative Control; PC is Positive Control; and S1 to S5 (sample locations) are the samples from the different locations sampled; 1000 bp ladder is molecular marker

Fig 8: Gel electrophoresis picture of *ace-1* amplicon of *Aedes aegypti*

Discussion:

Monitoring insecticide resistance is an important control strategy against malaria vectors and the resistance management strategies depend on the evidences of local vector resistance to insecticides (17). Insecticide resistance is a threat to vector borne disease control, and pyrethroids are still a widely used insecticide class in long-lasting insecticidal nets (LLINs) for malaria control (18,19). Pyrethroid resistance in the highly competent arbovirus vector *Aedes aegypti* is widely spread (20), and has also been confirmed in *A. albopictus* (20).

During the knockdown assessment for *Aedes* mosquitoes in this study more than half (>50%) of the mosquitoes were knocked down while that of bendiocarb and DDT was at 30 min for both insecticides. The present findings on bendiocarb and DDT mosquito knockdown time is however shorter than the findings by Ukpai and Ekedo (21) who reported that the highest KDT_{50} for bendiocarb and DDT was 50 minutes. Pirimiphos-methyl had longer KDR on the mosquitoes with <15% at 60 min. The results of the knockdown assessment showed that the tested insecticides induced knockdown of the adult *Aedes* mosquitoes (21-25). However, there is presence of knockdown resistance gene operating in populations of *Aedes* mosquitoes in the study locations (21,24,25). This could have been responsible for the level of resistance displayed by this mosquito to the various insecticides evaluated.

Out of all the insecticides the *Aedes* mosquitoes were exposed to, the highest knockdown efficacy was recorded with deltamethrin. At Orba, there was significant difference in the knockdown rate observed in deltamethrin (p> 0.05), at 10-20 min exposure, whereas other insecticides had significant differences (p<0.05) in knockdown rate at 40-60 min of exposure. Comparatively, knockdown rates of *Aedes* mosquitoes by deltamethrin across study sites did not differ significantly (p>0.05) for Obinagu, Neke and Ekwegbe Ulo, but differed significantly (p<0.05) at Umuopu.

The resistance *Aedes* mosquitoes to deltamethrin in the study locations, except for Ekwegbe Ulo, is quite strange due to the fact that deltamethrin belong to the subgroup of pyrethroids containing an alpha cyano-group in their chemical structure and are known for their potency against insect even at much lower concentration (26). However, resistance of *Aedes* mosquitoes to deltamethrin and other pyrethroids had been reported by Jirakanjanakit (27) and McAllister (28). The present findings therefore corroborate these earlier works. It is noteworthy that insecticidal programmes are already under jeopardy in these localities, except there is a quick and timely intervention. This is because of the growing resistance of *Aedes* mosquitoes in the study localities to the insecticides which have low mortality potency on them.

As deltamethrin is one of the most widely used insecticides for the control of *Aedes* mosquitoes (29,30), and their use in the study area could negatively affect the efficacy of vector control efforts there. Deltamethrin resistance could result from the widespread use of pyrethroids for the impregnation of bed nets and for indoor residual spraying against malaria vectors. Pyrethroid resistance has been recently reported in an *Aedes* mosquitoes' population in Ghana (31). Other studies have also reported pyrethroid resistance in *Aedes* mosquitoes in Africa and Asia (32-35).

Using the WHO (36) criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24-hour post exposure results indicated that the *Aedes* mosquitoes were largely resistant to the insecticides, except at Ekwegbe Ulo community that recorded mosquitoes' susceptibility to deltamethrin, bendiocarb and pirimiphos-methyl (100.0%). The present finding in Ekwegbe Ulo community is contrary to documented evidence on the resistance of mosquitoes to bendiocarb (37). Plausible explanation to this could be low use of carbamate-based insecticides in Ekwegbe Ulo as reported by Ukpai and Ekedo (21) in Umudike, unlike in Orba, Umuopu, Obinagu and Neke communities where resistance to these insecticides was observed. The shorter knockdown time of deltamethrin and bendiocarb observed from this study is traceable to the fact that both insecticides are synaptic poisons that inhibit the acetylcholinesterase enzyme in insects (38).

In this study, although pirimiphosmethyl had the longest knockdown time, it performed better in terms of mortality efficacy than the rest of the insecticides across the localities, except in Neke community that had higher mortality of mosquitoes for DDT. Hence, resistance to pirimiphos-methyl is not as high as those of DDT and the pyrethroids. The resistance to pirimiphos-methyl observed in the present study has been reported in several other geopolitical zones of Nigeria (39). The resistance observed with pirimiphos-methyl could be due to the fact that this insecticide was among the insecticides widely used for crop control in agriculture as reported by Odeyemi et al., (40) and the West Africa Productivity Programme (41) and could have exerted a high selection pressure on mosquito larvae. Also, whereas this study observed high resistance of *Aedes* mosq-

uitoes to organophosphate pirimiphos-methyl, the works of Ukpai and Ekedo (21) and Ibrahim et al., (24) reported a low resistance.

On the other hand, the *Aedes* mosquitoes were fully resistant to DDT across the study communities (<90%). Recent reports have suggested a contrasting pattern of DDT resistance across Nigeria, which is of great concern (10). The DDT resistance in *Aedes* mosquitoes is in tandem with previous studies (21,24,31,37). The high resistance of the mosquito to DDT goes a long way to prove the inefficacy of organochlorines in the control of *Aedes* mosquitoes in study locations. This is in agreement with other researchers who have lamented on the growing resistance of many mosquito species to DDT (42). Furthermore, this raises serious concern on the effectiveness of organophosphate insecticides in the study. Similar resistance to DDT was observed in both farm and non-farm sites in Lagos and Awka, Nigeria (43,44) as well as in Cameroun (45).

The resistance to DDT may be connected with the historical use of this insecticide in vector control activities in Nigeria. Also, DDT resistance in the study communities could be attributed to the heavy use of organochloride insecticides in the locations for agricultural purposes, which could have led to the development of resistance by mosquitoes to the organochloride class. This extensive use and abuse of DDT for agricultural purposes could have contributed immensely to the development of resistance in insect pests as earlier reported by Hadura (46). Indoor residual spraying (IRS) using DDT for malaria control has been reported to favour the selection of DDT resistance in *Anopheles* as well as *Aedes* vectors (45). The contamination of larval breeding places by insecticides used in agriculture has also been shown to select for DDT resistance in malaria vectors (45).

Conclusion:

This study showed that *Aedes* mosquitoes were widely resistant to all pesticides in Orba, Obinagu, Neke, and Umuopu; however, in Ekwegbe Ulo, the mosquitoes were susceptible to deltamethrin, bendiocarb, and pirimiphos-methyl. Since these insecticides are a major component of most vector control initiatives, this has significant implications for the management of malaria and its vectors in the Southeast Zone.

The effectiveness of long-lasting insecticide nets (LLINs), insecticide-treated nets (ITNs), and other vector control methods such indoor residual spraying (IRS) that may be used in the research region is seriously threatened. The National Malaria Control Programme would benefit from the data produced by this study when selecting an insecticide to be used in southeast Nigeria.

Contributions of authors:

NOC is involved in the study conceptualization and design; NOC and OOO were involved in the methodology and data analysis; NJO wrote the original draft of the manuscript; NOC, NJO, OOO, AN and NNR reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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Authors declare no conflict of interests.

Artificial Intelligence (AI) use disclosure

Artificial intelligence (AI) was used to format the abstract, introduction, materials and methods, results and discussion.

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Original Article **Open Access**

Prevalence of dermatophytoses among school children in a rural setting in Biase Local Government Area, Cross River State, Nigeria

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Abstract:

Background: Infections of the skin, nails and hair cause by dermatophytes species are common in both developing and developed countries, posing serious health challenge especially, among children of primary school age. This study is aimed at determining the prevalence and distribution of dermatophyte species among infected school children in Biase, Cross River State, Nigeria.
Methodology: This was a descriptive cross-sectional study of children with clinical features of dermatophytosis from 9 of the 55 government-approved primary schools in Biase local government area (LGA) of Cross Rives State, selected by stratified random sampling technique. All children in the 9 selected schools were first examined for dermatophytic lesions. Samples of the lesions were then collected and examined by direct microscopy, and also cultured on Dermatophyte Test Medium (DTM) and incubated aerobically at 26-29°C. Pure cultures of the isolates were further examined by macroscopy, microscopy and biochemical test reactions to phenotypically confirm the fungi identity.
Results: A total of 5946 primary school children were screened for dermatophytosis, 206 (3.5%) had clinically suggestive lesions, and 180 (3.0%) were mycologically confirmed to be dermatophytoses. The highest prevalence of infection was among children from the school in Obum/Etana (7.6%, n=17/223) while the least prevalence was Akpet central primary school (1.5%, n=24/1670) (x^2=43.94, p<0.0001). The prevalence of dermatophytoses was higher among school children in age group 8-10 years (3.3%, n=103/3114) and ≥ 11 years (3.3%, n=36/1096) than age group 5-7 years (2.4%, n=41/1736). The prevalence of infection among male school children (3.3%, n=100/2993) was slightly higher than female school children (2.7%, n=80/2953). A total of 9 species of dermatophytes were identified; *Trichophyton mentagrophytes, Trichophyton quinckeanum, Trichophyton violaceum*, *Trichophyton soudanense, Trichophyton tonsurans, Trichophyton schoenleinii*, *Microsporum canis*, *Microsporum gypseum* and *Microsporum audouinii,* with *T. soudanense* (59.1%, n=110) being the most frequently isolated while *T. tonsurans* (1.1%, n=2) was the least frequently isolated dermatophyte.
Conclusion: The prevalence of dermatophytoses among school children in Biase LGA of Cross River State, Nigeria is 3.0%, with high prevalence of infection among children in the age groups 8-10 and >11 years of age, and among parents/guardians that are low-income earners. Improved living condition of the people and standard hygiene practices may ameliorate the spread of dermatophytoses in the study setting.

Keywords: Dermatophytoses, Prevalence, School children; Rural setting.

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Prévalence des dermatophytoses chez les écoliers en milieu rural dans la zone de gouvernement local de Biase, État de Cross River, Nigéria

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Résumé:

Contexte: Les infections cutanées, unguéales et capillaires causées par des dermatophytes sont fréquentes dans les pays en développement comme dans les pays développés, et constituent un problème de santé publique majeur, notamment chez les enfants d'âge scolaire primaire. Cette étude vise à déterminer la prévalence et la répartition des espèces de dermatophytes chez les écoliers infectés de Biase, dans l'État de Cross River, au Nigéria.
Méthodologie: Il s'agit d'une étude descriptive transversale menée auprès d'enfants présentant des signes cliniques de dermatophytose, scolarisés dans 9 des 55 écoles primaires publiques de la zone de gouvernement local (LGA) de Biase, dans l'État de Cross River. Les écoles ont été sélectionnées par échantillonnage aléatoire stratifié. Tous les enfants des 9 écoles sélectionnées ont d'abord été examinés afin de rechercher des lésions dermatophytiques. Des prélèvements de ces lésions ont ensuite été effectués et examinés par microscopie directe, puis mis en culture sur milieu de test pour dermatophytes (DTM) et incubés en aérobiose à 26-29 °C. Les cultures pures des isolats ont ensuite été examinées par macroscopie, microscopie et tests biochimiques afin de confirmer phénotypiquement l'identité des champignons.
Résultats: Sur un total de 5946 élèves du primaire dépistés pour une dermatophytose, 206 (3,5%) présentaient des lésions cliniquement évocatrices et 180 (3,0%) ont reçu un diagnostic mycologique de dermatophytose. La prévalence la plus élevée d'infection a été observée chez les enfants de l'école d'Obum/Etana (7,6%, n=17/223), tandis que la plus faible a été enregistrée à l'école primaire centrale d'Akpet (1,5%, n=24/1670) (χ^2=43,94, p<0,0001). La prévalence des dermatophytoses était plus élevée chez les écoliers âgés de 8 à 10 ans (3,3%, n=103/3114) et de 11 ans et plus (3,3%, n=36/1096) que chez ceux âgés de 5 à 7 ans (2,4%, n=41/1736). La prévalence de l'infection chez les garçons (3,3%, n=100/2993) était légèrement supérieure à celle observée chez les filles (2,7%, n=80/2953). Au total, 9 espèces de dermatophytes ont été identifiées. Les dermatophytes suivants ont été identifiés: *Trichophyton mentagrophytes, Trichophyton quinckeanum, Trichophyton violaceum, Trichophyton soudanense, Trichophyton tonsurans, Trichophyton schoenleinii, Microsporum canis, Microsporum gypseum* et *Microsporum audouinii. T. soudanense* (59,1%, n=110) était l'espèce la plus fréquemment isolée, tandis que *T. tonsurans* (1,1%, n=2) était la moins fréquemment isolée.
Conclusion: La prévalence des dermatophytoses chez les écoliers de la zone de gouvernement local de Biase, dans l'État de Cross River (Nigeria), est de 3,0%. L'infection est particulièrement fréquente chez les enfants de 8 à 10 ans et de plus de 11 ans, ainsi que chez les parents/tuteurs à faibles revenus. L'amélioration des conditions de vie et des pratiques d'hygiène courantes pourrait contribuer à freiner la propagation des dermatophytoses dans la zone étudiée.

Mots-clés: Dermatophytoses, Prévalence, Écoliers Cadre rural.

Introduction

Dermatophytoses are superficial fungal infections caused by dermatophytes affecting the skin, hair and/or nails (1), and are generally termed tinea. Dermatophytes are filamentous fungi that invade and feed on keratinized tissue like skin, hair and nails, causing an infection (2). Dermatophytes are divided into three genera, of which *Trichophyton* (usually affecting skin, hair and nails), *Epidermophyton* (usually affecting skin) and *Microsporum* (usually affecting skin and hair) cause infection in humans. *Trichophyton rubrum* is the most common isolate observed in infections of the feet, body and nails.

Depending on the source of transmission, they are classified as anthropophilic, zoophilic and geophilic. They are clinically classified by infection site as tinea capitis (head), tinea faciei (face), tinea barbae (beard), tinea corporis (body), tinea manuum (hand), tinea cruris (groin), tinea pedis (foot) and tinea unguium (nails). Other clinical variants include tinea imbricata, pseudoimbricata, and Majocchi granuloma.

Globally, dermatophytoses affect approximately 20–25% of the population, with tinea capitis being one of the most common forms among school-aged children (3). The infection is especially prevalent in tropical and subtropical regions where warm temperatures, high humidity, and overcrowded living conditions promote the growth and transmission of dermatophytes (4-5). These fungi have worldwide distribution, and at present, there are 40 recognized species in the dermatophyte genera (6). Of these species about 25 species belonging to the genera *Epidermophyton, Microsporum* and *Trichophyton* are presently known to infect man (2).

Children of primary school age are usually between ages of 3 and 14 years who show high sensitivity to various pathogens including dermatophyte infections due to their close contact with the soil, and relatively low immune status compared to adults. Their inability to keep themselves from dirt due to their frequent contact with soil and infected pets animals makes this age group even more vulnerable. This study is therefore aimed at determining the prevalence of the infection and distributions of dermatophytes among rural school children in Biase Local Government Area (LGA), Cross River State, Nigeria.

Materials and method:

Study area:

Biase Local Government Area (LGA) is located at 96 km south of Calabar, the capital

city of Cross River State, Nigeria. The LGA is bounded in the North by Yakurr and Abi LGA and in the South and Southeast by Akamkpa and Odupk- pani LGAs respectively. On the West, it is bound by Abia State. Politically, the LGA falls within Southern senatorial district of the State and has a total land mass of 1,285.56km^3 with an area of 1.310km^3.

Biase also has a population of 169,183 (2006, census), with about half of the population (84, 598) in the age bracket of 0-19 years, at the time. Located within the tropical rainforest zone, the wet season of the LGA falls within the months of April and November of the year. It is predominantly a rural area and the major occupation of the people is subsistence agriculture with few farmers branching out into palm oil business and plantation farming.

Study design:

This was a community-based descriptive cross-sectional study, which allows for the determination of the prevalence of tinea capitis (scalp) and tinea corporis (skin) at a specific point in time among primary school children in the rural setting. This design also allowed for assessment of related risk factors associated with prevalence of dermatophytes infection among the study participants.

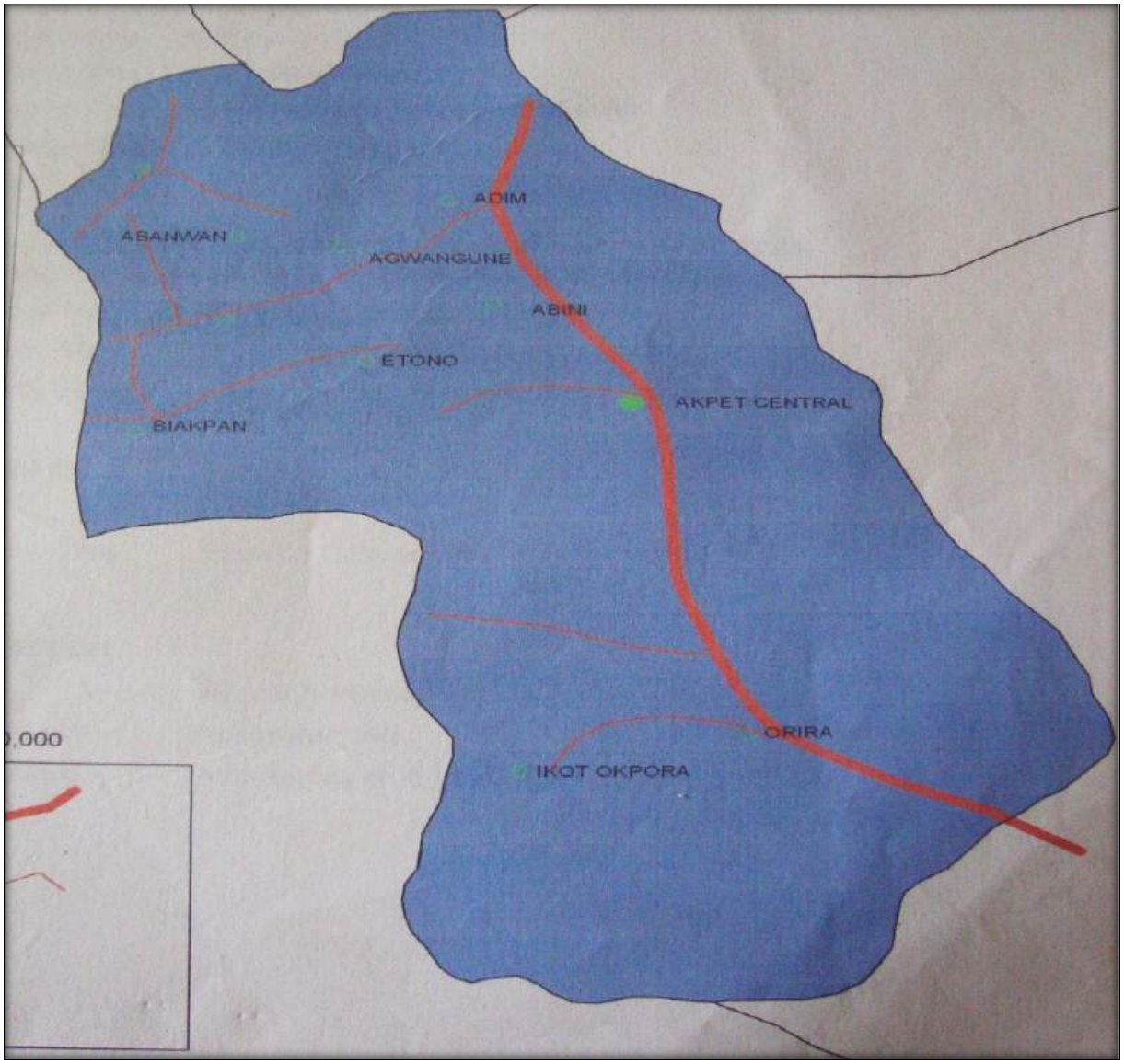

Fig 1: Map of Biase Local Government Area of Cross River State, Nigeria showing the geo-political wards selected for the study

Study population, sample size, and participants selection:

The sample size of 206 children with clinically suggestive dermatophytic lesions was determined using the formula described by Araoye (7). A total of 9 out of 55 government-approved primary schools in Biase LGA were selected for the study by stratified random sampling technique. All the children in the 9 selected schools were examined for dermatophytoses.

Lesions in children suggestive of dermatophytoses of the body include active, raised, scaly skin lesion with border that spread outward with central clearing, with accompanying itching/pruritus. Lesions in children with scalp infections include non-inflammatory patchy hair loss, scaling, dull brittle hair, 'black dots' where hair breaks; inflammatory purulent swelling with sinus tracts and hair loss; and favus. Children on antifungal or local medication two weeks before the commencement of the study, were excluded. The study was conducted during the rainy season (April to July 2024).

Ethical approval:

The field work commenced only after due approval from the Ethical Committee on Research of the Cross River State Ministry of Health (UCTH NHREC NO: NHREC/07/10/2012). Verbal consents of parents were obtained following assembly announcement by head teachers to the pupils, prior to the commencement of the study in all the 9 communities.

Data collection using a structured questionnaire:

A structured questionnaire was used to collect information on socio-demographic characteristics from the participants. The information includes sharing of personal belonging such as towels and combs, number of children per household, size of residential building, pet animals and access to basic social amenities.

We classified the socio-economic status of participants' parents as 'low income', 'average income' and 'above average income' earners based on sales output after a 4-day market cycle for petty farmers and traders, provision store owners, and businessmen, while others were assessed based on monthly salaries of civil and public servants.

Physical examination of participants for dermatophytic lesions:

Each child had a general examination conducted (head to foot) in good light to by trained nurses recruited from local health facility of selected communities, to identify children with suspected dermatophytic lesions who were then recruited based on the selection criteria for further examination.

Collection of specimens:

Each infected site was first cleaned with a sterile cotton wool soaked in 70% alcohol before collection of specimens. Skin and hair specimens were collected into pre-sterilized paper envelopes. Scales from skin lesions were collected by scraping outward with a blunt scalpel-blade from the edges of the lesion where most viable fungus was likely to be found. Specimen from scalp included hair stubs, the content of plugged follicles and skin scales. Specimens were appropriately labelled and transported to the microbiology laboratory of the University of Calabar Teaching Hospital, Calabar, for microbiological analysis.

Direct microscopic examination of specimens:

One to two fragments of hair or a portion of skin scrapings was placed in a drop of 10% or 20% potassium hydroxide (KOH) on a clean slide and a clean cover slip placed over it. The preparation was gently warmed by passing it over a flame several times. Dark pigmented hair required more time to allow digestion and clearing of the keratin for discerning it. For this reason, dark-haired specimens were allowed to stand for over 10 minutes. Microscopic examinations of each slide was done under low power (10x) and high power (40x) magnification to detect the presence of fungal elements as well as observe the type of hair infection exhibited.

Culture of specimens:

A portion of hair stubs or skin scrapings were inoculated on slopes of dermatophyte test medium (DTM, NEOGEN corp. USA). All slopes were incubated at room temperature (26-29^{o}C) and examined periodically every other day for growth. Each slope was allowed to stand for at least 21 days before discarded as negative. Contaminated cultures were sub-cultured into fresh media to separate dermatophytes from contaminants. All sub-cultures were incubated at room temperature for at least 3 weeks before discarding as negative.

Morphological and microscopic identification of dermatophytes:

Gross morphological characteristics and detailed microscopic features of each dermatophyte isolate were observed. The colonial characteristics noted included colour (surface and reverse), texture and topography. For microscopic features, a fragment of each colony was tease out on a clean slide with the aid of sterile pins on a drop of lactophenol cotton blue after which clean cover slip was placed on it. Each preparation was slightly warmed over a flame. The slide thus prepared was examined under the compound light microscope using both low

(10x) and high power (40x) magnification.

The characteristic features of the hyphae, large multicellular macroconidia and smaller unicellular microconidia of each isolate was observed and noted. Identification was based on the criteria enumerated by Rebell and Taplin (8), and Rippon (9).

Slide culture technique:

Slide culture using Potato Dextrose Agar (PDA, BBL) was set up for fungi that did not sporulate to facilitate their identification as described in Haley and Callaway (10).

Pure culture of dermatophytes and biochemical identification tests:

Pure cultures of every isolate were prepared before biochemical tests were performed. This was done by sub-culturing individual isolate onto fresh Sabouraud dextrose agar (SDA, BBL) without antibiotics and incubated at room temperature (26-29^{0}C) until pigment developed in front and/or back of colony or granular areas are observed. Subcultures were held for 10-14 days at which time lactophenol cotton blue preparations of fungal fragments were made and examined.

Urease test was used to further identify and differentiate between *Trichophyton mentagrophytes* and *Trichophyton rubrum* isolates. A slant of Christensen's urea agar was inoculated with a fragment of *T. mentagrophytes* colony and incubated at room temperature for 7 days. Medium turns bright pink which rapidly hydrolysed urea while the colour remained the same for suspected *T. rubrum* isolates.

Statistical analysis:

Data generated in the study were analyzed using SPSS version 20.0 (IBM Corporation, Armonk, New York, USA) for test of significance. Data were presented in tables, frequency and percentage. The Chi (x^2) square test was used to compare the prevalence of dermatophytoses between the different demographic characteristics, socio-economic status and school locations. P-value ≤ 0.05 was considered statistically significant.

Results:

A total number of 5946 primary school children (2993 males and 2953 females) from 9 localities in Biase LGA of Cross River State, Nigeria, were physically screened for dermatophytoses. Of these number, 206 (3.5%) children had clinically suggestive dermatophytic lesions, and 180 were mycologically-confirmed to be dermatophytoses by microscopy and/or culture, given a dermatophytoses prevalence of 3.0% in the study population (Table 1).

Table 1: Prevalence of suspected and confirmed dermatophytoses among the school children with respect to demographic characteristics in Biase LGA, Cross River State, Nigeria

Characteristics	No screened	No with clinical lesion	No with confirmed lesion*	x^2	*p* value
Age group (years)					
5-7	1736	51 (2.9)	41 (2.4)	3.700	0.1572
8-10	3113	118 (3.8)	103 (3.3)		
≥11	1096	37 (3.4)	36 (3.3)		
Sex					
Male	2993	112 (4.7)	100 (3.3)	1.813	0.1781
Female	2953	94 (2.2)	80 (2.7)		
Total	**5946**	**206 (3.5)**	**180 (3.0)**		

*Confirmed by positive microscopy and/or culture; x^2= Chi square

Table 2: Prevalence of dermatophytes infection among primary school children in Biase LGA Cross River State, Calabar, Nigeria

School location	No screened	No with clinical lesion	No with confirmed lesion*	Prevalence (%)
Ipene	300	16	13	4.3
Ibini	475	25	22	4.6
Obum/Etana	223	20	17	7.6^{+}
Agwagune	752	29	26	3.6
Urugbam	340	19	17	5.0
Ikot okpora	673	20	18	2.7
Abanwan	392	19	16	4.2
Biakpan	1121	30	26	2.3
Akpet central	1670	28	25	1.5^{++}
Total	**5946**	**206**	**180**	**3.0**

*Confirmed by positive microscopy and/or culture; x^2=43.94, p<0.0001; $^{+}$=highest prevalence; $^{++}$=lowest prevalence

The prevalence was higher among the male school children (3.3%, n=100/2993) than the female school children (2.7%, n=80/2953), but the difference was not statistically significant (x^2=1.813, p=0.1781). The prevalence was higher among school children in the age group 8-10 years (3.3%, n=103/3114) and age group ≥ 11yrs (3.3%, n=36/1096), compared to age group 5-7 years (2.4%, n=41/1736), but the difference was also not statistically significant (x^2=3.700, p<0.1572) (Table 1). The prevalence was highest for children in Obum/Etana locality (7.6%, n=17/223) and lowest for children in Akpet central (1.5%, n=25/1670) (x^2= 43.94, p<0.0001) (Table 2).

The distribution of clinical types of dermatophytoses by age of the children, presented in Table 3 shows that school children age group 5-7 years had significantly higher frequency of tinea capitis (81.8%, n=36/44) and lower frequency of tinea corporis (18.2%, n=8/44), compared to children in age group 8-10 years (57.4%, n=61/106 vs 42.5%, n = 45/106) and age group ≥11 years (60.0%, n=18/30 vs 30.0%, n=12/30) (x^2=8.175, p<0.0168).

Table 3: Clinical types of dermatophytoses with respect to age and gender of children in Biase LGA, Cross River State, Nigeria

Characteristics	Tinea capitis (%)	Tinea corporis (%)	Total	x^2	p value
Age group (years)					
5-7	36 (81.8)	8 (18.2)	41	8.175	0.0168*
8-10	61 (57.4)	45 (42.5)	103		
≥11	18 (60.0)	12 (40.0)	36		
Sex					
Male	72	28 (28.0)	100	2.220	0.136
Female	43	37 (46.2)	80		
Total	**115 (63.9)**	**65 (36.1)**	**180**		

x^2=Chi square test; *=statistically significant

Table 4: Distribution of the dermatophyte isolates according to the sites of infection in school children from Biase LGA, Cross River State, Nigeria

Isolates	Tinea capitis (%)	Tinea corporis (%)	Total (%)
Trichophyton mentagrophytes (Z)	6 (5.0)	20 (30.8)	26 (14.0)
Trichophyton quinckeanum (Z)	0	6 (9.2)	6 (3.2)
Trichophyton violaceum (A)	7 (5.8)	8 (12.3)	15 (8.1)
Trichophyton soudanense (A)	89 (73.6)	20 (30.8)	110 (59.1)
Trichophyton tonsurans (A)	2 (1.7)	0	2 (1.1)
Trichophyton schoenleinii (A)	8 (6.6)	4 (6.2)	12 (6.5)
Sub-total	**112 (65.9)**	**58 (34.1)**	**170 (91.4)**
Microsporum canis (Z)	0	3 (4.6)	3 (1.6)
Microsporum gypseum (G)	1 (0.8)	4 (6.2)	5 (2.7)
Microsporum audouinii (A)	8 (6.6)	0	8 (4.3)
Sub-total	**9 (7.4)**	**7 (10.8)**	**16 (8.6)**
Total	***121 (65.0)**	**65 (35.0)**	***186 (100.0)**

*=Additional 6 isolates were identified from mixed infections; A = Anthropophiles; G=Geophiles; Z=Zoophiles

The distribution of dermatophytes species according to clinical sites of infection presented in Table 4 showed that 65.0% (n=121/186) of dermatophyte species were isolated as causal agent of tinea capitis compared to 35.0% (n=65/186) for tinea corporis. *T. soudanense* was the principal agent responsible for 73.6% (n=89/121) of tinea capitis, followed by *M. audouinii* (6.6%, n=8/121), *T. schoenleinii* (6.6%, n=8/121), *T. violaceum* (5.8%, n=7/121), *T. mentagrophytes* (5.0%, n=6/121), *T. tonsurans* (1.7%, n=2/121) and *M. gypseum* (0.8%, n=1/121). Similarly, *T. mentagrophytes* (30.8%, n=20/65) and *T. soudanense* (30.8%, n=20/65) were the major etiologic agents of tinea corporis, followed by *T. violaceum* (12.3%, n=8/65), *T. quinckeanum* (9.2%, n=6/65), *T. schoenleinii* and *M. gypseum* (6.2%, n=4/65) and *M. canis* (4.6%, n=3/65).

Out of the total 9 dermatophyte species isolated, *T. soudanense* was the most frequent agent (59.1%, n=110/186) identified, followed by *T. mentagrophytes* (14.0%, n=26/186) while *T. tonsurans* was the least frequent species isolated (1.1%, n=2/186). Generally, 91.4% (n=170/186) of the dermatophyte isolates belonged to the *Trichophyton* genera while only 8.6% (n=16/186) belonged to the *Microsporum* genera in the study. Of the 9 dermatophyte species isolated, 5 were anthropophilic, 3 were zoophilic and 1 was geophilic.

Table 5 shows the socioeconomic status of parents in relations to the infected school children based on monthly income per capital. The overall prevalence of dermatophytes infection was higher among children whose parents /guardians were low-income earners (73.3%, n=132/180) than the average income earners (26.7%, n=48/180), and this trend occurred across the 9 localities, while none of the parents/guardians of the school children were above average income earners.

Fig 2 is the picture of a flat colony of *T. mentagrophytes* with fine saw teeth isolated from skin of infected children. Fig 3 is a picture of a small white, fluffy bottoned-smooth walled, blunt ended macroconidia up to 6 cells on Lactophenol Cotton Blue (LCB) stain. Fig 4 is a picture of diffuse alopecia with pronounced scaliness caused by *T. mentagrophytes.* Fig 5 is a picture of erythematous lesions showing central clearing caused by *M. canis.*

Table 5: Socio-economic status of parent/guardian of school children with dermatophytoses in the locality based on monthly earning per household

School location	No with confirmed lesion[+]	Actual earnings per month (in USD) for affected household		
		Low income*	Average income**	Above average income***
Ipene	13	10 (76.9)	3 (23.1)	0
Ibini	22	17 (77.3)	5 (22.7)	0
Obum/Etana	17	13 (76.5)	4 (23.5)	0
Agwagune	26	20 (76.9)	6 (23.1)	0
Urugbam	17	11 (64.7)	6 (35.3)	0
Ikot okpora	18	12 (66.7)	6 (33.3)	0
Abanwan	16	10 (62.5)	6 (37.5)	0
Biakpan	26	21 (80.8)	5 (19.2)	0
Akpet central	25	18 (72.0)	7 (28.0)	0
Total	**180**	**132 (73.3)**	**48 (26.7)**	0

[+]=Confirmed by positive microscopy and/or culture; One US dollar (1$) = ₦1,424.14 (rate as at March 2024); *=Low-income earners: ₦30,000 - ₦100,000 per month;
=Average income earners: ₦100,000 - ₦250,000 per month; *= Above average income earners: ≥ ₦250,000 per month

Fig 2: Flat colony of *Trichophyton mentagrophytes* with fine saw teeth isolated from skin of infected children

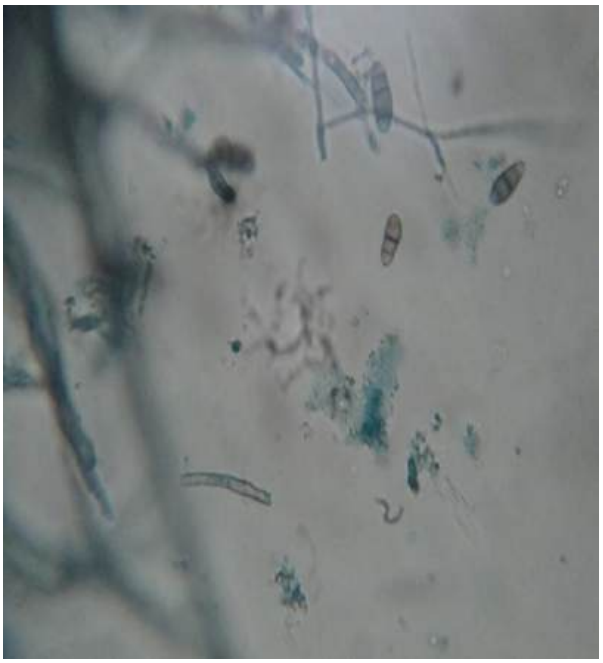

Fig: 3 shows a small white, fluffy bottoned-smooth walled, blunt ended macroconidia up to 6 cells on lactophenol cotton blue (480x). Snapshot, taken using a digital camera (Sharp product, USA) via the binoculars

Fig 4: Diffused alopecia with pronounced scaliness caused by *Trichophyton mentagrophytes*

Fig 5: Erythematous lesions showing central clearing caused by *Microsporum canis.*

Discussion:

Infections of the skin, nails and scalp cause by dermatophytes species are common in developing and developed countries alike. As reported by Rodriguez-Cerdeira et al., (11), the frequency of the infection ranges from 0.4% to 87.7% in Africa, 0.2% to 74.0% in America, 0.04% to 78.6% in Europe, and 0.01% to 91.2% in Asia. However, the species involved and the resulting clinical entities vary both geographically and with time. This study reports the prevalence of dermatophyte infection of 3.0% in a community-based study of school children in a local government area of Cross River State, Nigeria, with the highest prevalence of 7.6% in a school at Obum/Etana and lowest prevalence of 1.5% in a school at Akpet central. The prevalence in this study is lower than the study by Anosike et al., (12) in Northern Ebonyi State who reported a 21.1% prevalence of the infection among primary school pupils and by Ndako et al., (13) in Nasarawa LGA of Kano State, who also reported higher prevalence of 58.2% among children in the same age group.

The most frequent clinical type of dermatophyte infection among the school children was tinea capitis, which accounted for 63.9% and was observed to be more frequent in children aged 8-10 years and ≥11 years old. This agrees with the studies of Ajao and Akintunde (14), and Egere and Gugnani (15) conducted in the 1980s, which reported that tinea capitis was most frequent among children aged 5 to 10 years. Although the prevalence of dermatophytoses was higher among male (3.3%) than female (2.7%) children in our study, there was no significant association of the infection with gender (p=0.1781).

Of the 9 dermatophyte species isolated, 5 were anthropophilic, 3 was zoophilic and 1 geophilic. The slightly higher frequency of anthropophilic species is attributed to such factors as sharing of personal items like combs, towels, pillows, hairbrushes, unsterilized clippers from barbers' shop and body contacts, which facilitates person-to-person transmission. Furthermore, more, majority of the dermatophytes belong to the *Trichophyton* genera (91.4%) while *Microsporum* genera only constituted 8.6%. Although, no member of the genus *Epidermophyton* was identified in the study, species belonging to this genus have been recovered from lesions of tinea corporis and tinea pedis from studies conducted in various part of Nigeria, including Enugu, Kano, Abakaliki and Lagos state (16-17).

Trichophyton soudanense was the predominant etiologic agent of tinea capitis constituting 73.6%, (n=89) of the total isolates causing scalp infection, confirming an earlier study by Ekanem and Gugnani (18) which identified *T. soudanense* both as the most prevalent of all dermatophyte isolates (54.7%) in the old Cross River State and the principal etiologic agent of tinea capitis. *Microsporum audouinii* accounted for only 6.8% of the dermatophytes isolates responsible for the infection of scalp, with all occurring among the male school children. Further, low prevalence of tinea corporis in the riverine area of Agwagune caused by *M. canis* (4.6%, n=3/65) was recorded in this study. This may be linked to frequent association of children with infected pet animals such as dogs and cats.

A previous study by Ndako et al., (13) in Kano, Northern Nigeria reported 15.4% prevalence of *M. canis* while the same organism was reported as the most frequently isolated agent of tinea capitis in most of Central and Southern Europe, Puerto-Rico, and Saudi Arabia (19-22), affirming the impact of geographical location on the epidemiology of the infection. *Microsporum gypseum* on the other hand, was the only geophilic species encountered in this study and represented 2.7% of dermatophytes isolates identified. The 5 cases involving this specie occurred among school children in St. Martin's primary school Ibini and community primary school Obum/Etana probably due to their constant and direct interaction with soil. These two localities were the most underdeveloped of all the rural settings investigated compared to Biakpan and Akpet central that had features of an emerging sub-urban area, which may explain why the latter had lower prevalence of infection in the study.

Six cases of mixed infections were recorded in this study, representing 3.2% of dermatophytes isolates, out of which 5 cases were mixed infection involving *T. soudanense* and *T.* violaceum; *T. soudanense* and *M. audouinii,* and *T. violaceum* and *T. schoenleinii.* Majority of the population affected by this disease came from household with low-income earnings, linking poverty and lack of basic social amenities to the burden of the infection in rural settings.

The major limitation in this study is the descriptive cross-sectional design that cannot establish cause-effect relationship between risk factors and dermatophytosis as only association can be determined. Another limitation is that no molecular confirmation of dermatophytes was performed, which may lead to misidentification with the phenotypic methods used.

Conclusion:

The prevalence of dermatophytoses in school children in Biase LGA of Cross River State, Nigeria is 3.0%, with high prevalence of infection among children in the age groups 8-10 and >11 years of age, and among parents/ guardians that are low-income earners. Improved living condition of the people and standard hygiene practices may ameliorate the spread of dermatophytoses in the study setting.

Contributions of authors:

UII and AL were involved in the conceptualization, design and writing of the manuscript; UII was involved in data collection and analysis of results; Ethical approval of the study was facilitated by UAA. AL supervised the research work. UAA and AJ edited the manuscript. All authors read and approved the manuscript for publication.

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Conflict of interest:

No conflict of interest is declared.

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Original Article **Open Access**

Multidrug and colistin resistance in *Enterobacter* species isolated from water, human, and animal faecal samples in selected rural communities in Akwa Ibom State, Nigeria

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Abstract:

Background: Infection caused by antibiotic resistant *Enterobacter* spp. is a growing public health challenge as this significantly contributes to disease burden and high mortality in resource-limited settings. This study aimed to determine multidrug and colistin resistance patterns of *Enterobacter* isolates from water, human and animal samples in six rural communities in Akwa Ibom State, Nigeria.
Methodology: This was a laboratory-based study of 124 non-duplicate *Enterobacter* species isolated from water (n=43), human (n=46), goat (n=22) and dog (n=13) faecal samples in six local government areas (LGAs) of Akwa Ibom State. *Enterobacter* species were isolated from the samples following aerobic culture on Violet Red Bile Glucose agar after preliminary incubation in Enterobacteriaceae Enrichment broth at 37°C for 18 hours. Identification of *Enterobacter* isolates to species level was done using VITEK®2 compact system. Antibiotic susceptibility testing (AST) of isolates to selected antibiotics was performed using the Kirby-Bauer disc diffusion method, while colistin susceptibility testing (CST) was performed using disk elution MIC method. Multidrug resistance (MDR) was defined as resistance of an isolate to antibiotics in three or more classes. Multiple antibiotic resistance (MAR) index was determined as the number of antibiotics to which an isolate was resistant to, divided by the number of antibiotics to which the isolate was tested.
Results: Of the 124 non-duplicate *Enterobacter* isolates, 8 species were identified; *E. cloacae* (n=42), *E. aerogenes* (n=41), *E. cloacae* subsp *dissolvens* (n=17), *E. gergoviae* (n=11), *E. quasiroggenkampii* (n=4), *E. mori* (n=3), *E. hormaechei* (n=3), and *E. bugandensis* (n=3). *Enterobacter* spp from water samples were 95.3% and 90.7% sensitive to ciprofloxacin and ceftazidime respectively; those from humans were 95.7% sensitive to amoxicillin-clavulanate, those from goats were 86.4% sensitive to ceftazidime while those from dogs were 92.3% sensitive to amoxicillin-clavulanate and 84.6% sensitive to doxycycline and ceftazidime. The isolates exhibited varying resistance to imipenem, cefoxitin, and streptomycin. *Enterobacter* isolates from water, human, goat and dog samples had MARI of > 2 in 34.9%, 60.9%, 27.3% and 46.2% respectively, with resistance to 2-6 antibiotics. The MDR rate among *Enterobacter* isolates was 34.7% (n=43), with 30.2%, 41.3%, 27.3% and 38.5% in isolates from water, human, goat and dog samples respectively. Colistin-resistant rates in *Enterobacter* isolates from water, human, goat and dog samples were 25.6%, 28.3%, 27.3% and 38.5%, respectively, with *E. cloacae, E. aerogenes* and *E. dissolvens* being the most colistin-resistant isolates. *Mcr-1* gene was detected in colistin-resistant isolates from samples in two of the six rural communities (Ikot Abasi and Uruan) in the State.
Conclusion: The findings of this study underscore the need for continuous surveillance to monitor resistance patterns and implementation of antibiotic stewardship and infection control policies to prevent the emergence and spread of resistant strains in the community.

Keywords: Colistin resistance, *Enterobacter* spp., Multidrug-resistance, water, faecal, samples

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Résistance multidrogue et colistine chez les espèces d'*Enterobacter* isolées à partir d'échantillons d'eau, de matières fécales humaines et animales dans certaines communautés rurales de l'État d'Akwa Ibom, Nigeria

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Résumé:

Contexte: Infection causée par Enterobacter spp. Il s'agit d'un défi de santé publique croissant dans la mesure où cela contribue de manière significative à la charge de morbidité et à une mortalité élevée dans les contextes aux ressources limitées. Cette étude visait à déterminer les profils de résistance aux médicaments et à la colistine des isolats d'Enterobacter provenant d'échantillons d'eau, d'humains et d'animaux dans six communautés rurales de l'État d'Akwa Ibom, au Nigeria.
Méthodologie: Il s'agissait d'une étude en laboratoire portant sur 124 espèces d'Enterobacter non dupliquées isolées d'échantillons fécaux d'eau (n=43), d'humains (n=46), de chèvres (n=22) et de chiens (n=13) dans six zones de gouvernement local (LGA) de l'État d'Akwa Ibom. Les espèces d'Enterobacter ont été isolées des échantillons après culture aérobie sur gélose Violet Red Bile Glucose après incubation préliminaire dans un bouillon d'enrichissement Enterobacteriaceae à 37°C pendant 18 heures. L'identification des isolats d'Enterobacter au niveau de l'espèce a été réalisée à l'aide du système compact VITEK®2. Les tests de sensibilité aux antibiotiques (AST) des isolats à des antibiotiques sélectionnés ont été réalisés à l'aide de la méthode de diffusion sur disque de Kirby-Bauer, tandis que les tests de sensibilité à la colistine (CST) ont été réalisés à l'aide de la méthode CMI par élution sur disque. La multirésistance aux antibiotiques (MDR) a été définie comme la résistance d'un isolat à des antibiotiques appartenant à trois classes ou plus. L'indice de résistance multiple aux antibiotiques (MAR) a été déterminé comme le nombre d'antibiotiques auxquels un isolat était résistant, divisé par le nombre d'antibiotiques auxquels l'isolat a été testé.
Résultats: Parmi les 124 isolats d'Enterobacter non dupliqués, 8 espèces ont été identifiées; *E. cloacae* (n=42), *E. aerogenes* (n=41), *E. cloacae* subsp *dissolvens* (n=17), *E. gergoviae* (n=11), *E. quasiroggenkampii* (n=4), *E. mori* (n=3), *E. hormaechei* (n=3) et *E. bugandensis* (n=3). Les espèces d'*Enterobacter* provenant des échantillons d'eau étaient respectivement sensibles à 95,3% et 90,7% à la ciprofloxacine et à la ceftazidime; ceux provenant d'humains étaient sensibles à 95,7% à l'amoxicilline-clavulanate, ceux provenant de chèvres étaient sensibles à 86,4% à la ceftazidime tandis que ceux provenant de chiens étaient sensibles à 92,3% à l'amoxicilline-clavulanate et à 84,6% à la doxycycline et à la ceftazidime. Les isolats présentaient une résistance variable à l'imipénème, à la céfoxitine et à la streptomycine. Les isolats d'*Enterobacter* provenant d'échantillons d'eau, d'humains, de chèvres et de chiens présentaient un MARI > 2 dans 34,9%, 60,9%, 27,3% et 46,2% respectivement, avec une résistance à 2 à 6 antibiotiques. Le taux de MDR parmi les isolats d'*Enterobacter* était de 34,7% (n=43), avec 30,2%, 41,3%, 27,3% et 38,5% dans les isolats provenant d'échantillons d'eau, d'humains, de chèvres et de chiens, respectivement. Les taux de résistance à la colistine dans les isolats d'Enterobacter provenant d'échantillons d'eau, d'humains, de chèvres et de chiens étaient respectivement de 25,6%, 28,3%, 27,3% et 38,5%, *E. cloacae, E. aerogenes* et *E. dissolvens* étant les isolats les plus résistants à la colistine. Le gène *mcr-1* a été détecté dans des isolats résistants à la colistine provenant d'échantillons provenant de deux des six communautés rurales (Ikot Abasi et Uruan) de l'État.
Conclusion: Les résultats de cette étude soulignent la nécessité d'une surveillance continue pour surveiller les profils de résistance et la mise en œuvre de politiques de gestion des antibiotiques et de contrôle des infections afin de prévenir l'émergence et la propagation de souches résistantes dans la communauté.

Mots-clés: Résistance à la colistine, *Enterobacter* spp, multirésistance aux antibiotiques, eau, matières fécales, échantillons

Introduction:

Antimicrobial resistance (AMR) is a growing global public health threat. It is particularly worrisome among *Enterobacter* spp and other Gram-negative bacteria (GNB) as they contribute significantly to disease burden and mortality (1). In 2019, it was reported that AMR directly caused an estimated 1.27 million deaths, and an additional 5 million deaths were associated with *Enterobacter* spp (2). A major concern is the rise of drug-resistant infections, especially those caused by antibiotic-resistant bacteria acquired in hospitals, which are becoming more common in developing countries, including Nigeria (3).

Multidrug-resistant (MDR) *Enterobacter* isolates have emerged and spread worldwide due to widespread use of antibiotics (4). These organisms seem to have innate resistance to older antimicrobial agents and have the propensity to rapidly develop resistance to newer antimicrobial agents (1). Treatment of infections with *Enterobacter* spp is difficult because of resistance to third generation cephalosporins, penicillin and quinolones (5). As reported by Davin-Regli et al., (6), *Enterobacter* spp naturally carries genes encoding AmpC β-lactamases, leading to intrinsic resistance to ampicillin, amoxicillin, first-generation cephalosporins and cefoxitin. These pathogens can adapt to the environment and acquire various mobile gene-

tic elements (MGEs) coding for resistance, making them multidrug-resistant (6). The emerging resistance of *Enterobacter* to β-lactams and carbapenem antibiotics makes treatment more challenging, especially in resource limiting countries (1).

To curtail the rising incidence of infections caused by MDR *Enterobacter* spp, healthcare systems have increasingly turned to colistin, which is regarded as a last-line therapeutic option for the treatment of severe infections caused by MDR Gram-negative bacteria (GNB), including *Enterobacter* spp (1). Colistin is a polycationic antibiotic with broad-spectrum bactericidal activity. It targets the lipopolysaccharides in the bacterial cell walls, disrupting their outer membrane and causing the bacteria to break apart and die (7). Colistin was initially restricted to veterinary medicine due to its nephrotoxicity and neurotoxicity (8). However, the rise of MDR and carbapenem-resistant *Enterobacter* has led to its renewed use in humans (9).

Unfortunately, the increasing resistance to this critical antibiotic is becoming a global challenge, as it is becoming less effective in treating resistant infections, making patient care even more difficult (10). Understanding the patterns of antibiotic resistance and monitoring colistin-resistant *Enterobacter* isolates are essential for developing effective treatment and intervention strategies to prevent their spread in various environments, including hospitals and community settings. However, there is paucity of comprehensive data on multidrug and colistin resistance in environmental, human and animal isolates of *Enterobacter* spp. in Akwa Ibom State. Therefore, this study was carried out to determine the antibiotic susceptibility, multidrug resistance profiles and colistin resistance rates in *Enterobacter* spp isolated from water, human and animal samples in selected rural communities of Akwa Ibom State, Nigeria.

Materials and method:

Study design and settings:

This was a laboratory-based study of *Enterobacter* spp isolated from water, human and animal in rural communities of Akwa Ibom State. Nigeria. The State covers a total land area of 7,249 square kilometer and has a population of 5,451,277 (11). Akwa Ibom lies between latitude 4º32” and 5º33” North and longitude 7º35" and 8º25" East. The State is bordered on the East by Rivers State, on the West by Cross River State, on the North by Abia State and on the South by the Gulf of Guinea.

The people of Akwa Ibom State are culturally homogeneous with a common identity and are reputed to be the first settlers in the present-day southeastern Nigeria. The three major dialects are the Ibibio, Anang and Oron. Other subgroups include Eket, Ibeno, Itu Mbuso and the Andonis. The six rural communities selected for study using random sampling technique included Ikot Ntuen in Ikono Local Government Area (LGA), Ikot obong in Ibiono Ibom LGA, Nto Edino in Obot Akara LGA, Issiet Ekim in Uruan LGA, Isong Inyang in Mbo LGA and Ikot Atan in Ikot Abasi LGA.

Ethical consideration:

Ethical approval was requested and obtained from Research Ethics Committee of Akwa Ibom State Ministry of Health Research Ethics Committee (AKSMHREC) with HREC assigned (AKHREC/20/04/23/145). Since the study also involved direct collection of specimens from human and animal subjects, written informed consent was obtained from the participants and animal owners prior to their inclusion and sample collection.

Collection and transportation of samples:

Samples collected for analysis include water, human faecal, goat faecal and dog faecal samples. Water samples were collected at targeted stream points directly into 500ml sterilized polypropylene bottles. At the sampling point, the bottles were opened aseptically and rinsed with the stream water and held at a right angle and submerged to a depth of about 20cm with the mouth of the bottle facing upwards directly opposite the current of the flowing stream and filled to the brim. The bottles were stored in ice packed coolers. In-situ physicochemical analyses were carried out using water scooper to transfer the water into a clean bucket where the actual measurement was taken, some part of the water was also taken in another 500ml container and labeled for further biochemical and physicochemical analysis.

Human faecal samples were collected from willing volunteers at the nearby primary healthcare centers within the community. Prior to sample collection, they were educated on how to collect their faecal samples using sterile widemouth, screw cap leak proof sample containers. Animal faecal samples from goats and dogs were collected within the six communities using sterile swab-sticks. Faecal samples were stored in ice-coolers and transported to Microbiology Laboratory, University of Uyo, Nigeria for processing.

Isolation and identification of *Enterobacter* from samples

For the isolation of *Enterobacter* spp. from water, animal and human faecal samples. One mil (1ml) of water sample and one gram (1g) of each faecal samples from human, goat and dog was aseptically inoculated into sterile peptone water and then inoculated into 9 mL of Enterobacteriaceae Enrichment (EEE) broth (Himedia, India). The enrichment cultures were incubated at 37°C for 18 hours, after which 1 ml of each pre-enriched culture was inoculated onto Violet Red Bile Glucose Agar (VRBGA, Himedia, India) by pour plating and incubated at 37°C for 24 hours. Colonies appearing as red or purple with a surrounding halo on VRBGA were presumptively identified as *Enterobacter* spp (12). All presumptive isolates were confirmed by VITEK®2 identification compact system (BioMérieux, Marcy-L'Étoile, France).

Antibiotic susceptibility testing:

The antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method on Mueller Hinton Agar (MHA, Oxoid™ Thermo scientific ™ UK) plates. A loopful of the pure isolate was prepared and adjusted to 0.5 McFarland standard (equivalent to 10^8 CFU/ml) and spread on the surface of the agar using a sterile hockey stick. Nine selected antibiotic disks (Bioanalyse(R) Venimahalle Ankara/Turkey) were used and this included imipenem (IPM, 10µg), meropenem (MEM, 10µg), doxycycline (DOX, 30µg), ceftazidime (CAZ, 30µg), ciprofloxacin (CIP, 5µg), amoxicillin-clavulanate (AMC, 30µg), cefoxitin (FOX, 30µg), Streptomycin (S, 10µg) and trimethoprim/sulfamethoxazole (SXT, 23.75µg/1.25µg). The disks were gently placed on the agar plates, and incubated at 37°C for 24 hours. The diameters of the zones of inhibition (in mm) were measured with a meter ruler and interpreted as resistant, intermediate or sensitive in accordance with the 2024 CLSI guidelines and interpretative criteria (13). Multidrug resistance was defined as resistance of an isolate to antibiotics in three or more classes (14).

Colistin susceptibility testing by broth disk elution MIC method:

Colistin was tested by colistin broth disk elution (CBDE) test on cation-adjusted Mueller Hinton Broth (CA-MHB; Himedia laboratories, Mumbai, India) according to the CLSI 2024 guidelines (13,15). For each isolate, 0, 1, 2 and 4 10-µg colistin disc was added to four tubes containing 10 ml each of CA-MHB to achieve final concentrations of 0 (control growth), 1, 2, and 4 µg/ml in the CA-MHB tubes. The tubes were vortexed and left at room temperature for 30 mins to allow the colistin to adequately elute from the discs.

To produce a standardized inoculum, 3-5 colonies from 24 hours agar plate were suspended in 4-5 ml of sterile saline. The turbidity was adjusted to match that of a McFarland 0.5 standard. To achieve a final inoculum concentration of $7.5x10^5$ CFU/ml, 50µL aliquot of the standardized inoculum was added to each tube. MIC values were read as the lowest concentration that completely inhibits growth of the isolate after 16-20 hours incubation at 35°C. Interpretation of result was done using the CLSI breakpoints of ≤2µg/mL as intermediate and ≥4µg/mL as resistant (13).

Determination of multiple antibiotics resistant index (MARI) and multidrug-resistant (MDR) profile of *Enterobacter* spp.:

Multiple antibiotic resistance (MAR) index was determined using the formula MAR= x/y, where 'x' was the number of antibiotics to which the test isolate displayed resistance and 'y' was the total number of antibiotics to which the test isolates were evaluated for sensitivity. MARI >2 indicates that the isolates are from an environment where antibiotics are widely used (16). Multiple antibiotic resistance was also described by determining multidrug-resistance (MDR) profiles of the isolates as defined by Magiorakos et al., (14).

Amplification of colistin resistance gene:

The *mcr-1* gene of the isolates were amplified using the *mcr-1F:*5'-GTCGGTATGCTC GTTGGCTTAG-3and Mcr1R: 5'-CATAGGCATTGC TGTGCGTCTG-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40µl for 35 cycles. The PCR mix included the X2 Dream Taq Master mix (Inqaba Biotec, South Africa), Taq polymerase, DNTPs, $MgCl_2$, primers at a concentration of 0.5µM and the extracted DNA as template.

The PCR conditions were as follows; initial denaturation at 95ºC for 5 mins, denaturation at 95ºC for 30 seconds, annealing at 54ºC for 30 seconds, extension at 72ºC for 30 seconds for 35 cycles, and final extension at 72 ºC for 5 mins. The product was resolved on a 1% agarose gel at 130V for 30 mins and visualized on a blue light transilluminator.

Statistical analysis:

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, PASW–Predictive Analytics Software, version 25.0). Antimicrobial resistance patterns of *Enterobacter* spp, multiple antibiotic resistance (MAR) indices, multi-drug resistance, and colistin resistance were determined using descrip-

tive statistics. Associations between categorical variables were evaluated using the Chi-square (χ^2) test, and differences were considered statistically significant at $p<0.05$.

Results:

***Enterobacter* species isolated from samples:**

A total of 124 non-duplicate *Enterobacter* spp were isolated from water (n=43), human (n=46), goat (n=22) and dog (n=13) faecal samples. A total of 8 species of *Enterobacter* were identified; *E. cloacae* (n=42), *E. aerogenes* (n=41), *E. cloaca* subsp *dissolvens* (n=17), *E. gergoviae* (n=11), *E. quasiroggenkampii* (n=4), *E. mori* (n=3), *E. hormaechei* (n=3), and *E. bugandensis* (n=3).

Antibiotic susceptibility of *Enterobacter* isolates:

Enterobacter cloacae isolated from water samples were highly sensitive to amoxicillin/ clavulanate (86.7%) and ceftazidime (86.7%), and 66.7% were sensitive to ciprofloxacin, but 53.3% of the isolates were resistant to cefoxitin and streptomycin. *Enterobacter aerogenes* were sensitive to amoxicillin/clavulanate (92.3%), ceftazidime (84.6%) and trimethoprim/sulfamethoxazole (84.6%). However, 46.1% of the isolates were resistant to cefoxitin and streptomycin. All *E. mori* were highly sensitive to ceftazidime, ciprofloxacin, amoxicillin/ clavulanate and trimethoprim/sulfamethoxazole. About 90.7% of the *Enterobacter* spp from water samples were sensitive to ceftazidime and 74.4% to amoxicillin/clavulanate while they were resistant to streptomycin (37.2%), imipenem (25.6%) and meropenems (27.9%) (Table 1).

The antibiotic susceptibility profile of *Enterobacter* spp isolated from human faecal samples result indicates that *E. aerogenes* were sensitive to amoxicillin/clavulanate (88.9%), but 50.0% of the isolates were resistant to streptomycin. *Enterobacter gergoviae* was sensi tive to amoxicillin/clavulanate (100.0%), ceftazidime (66.7%) and trimethoprim/sulfamethoxazole (66.7%), and resistant to cefoxitin and streptomycin (50.0%). Overall, *Enterobacter* spp from human faecal samples were sensitive to amoxicillin/clavulanate (95.7%) and trimethoprim/sulfamethoxazole (76.1%) while 39.1% of the isolates were resistant to doxycycline and (34.8%) to streptomycin (Table 2).

All *E. cloacae* from dogs were sensitive to amoxicillin/clavulanate and trimethoprim/ sulfamethoxazole but 50.0% of the isolates were resistant to imipenem and meropenem. All *E. hormaechei* were sensitive to meropenem, doxycycline and ceftazidime but were resistant to all other antibiotics. All *E. quasiroggenkampii* were sensitive to doxycycline, ceftazidime, ciprofloxacin and amoxicillin/clavulanate. Overall, *Enterobacter* spp from dog samples were sensitive to amoxicillin/clavulanate (92.3%), doxycycline and ceftazidime (84.6%) (Table 3).

Enterobacter aerogenes from goat samples were highly sensitive to all the antibiotic used except ciprofloxacin. About 66.7% of *E. cloacae* subsp *dissolvens* were resistant to meropenem, cefoxitin and streptomycin. *E bugandensis* was sensitive to doxycycline, ceftazidime, ciprofloxacin, amoxicillin/clavulanate and trimethoprim/sulfamethoxazole. All *E. hormaechei* from goat samples were sensitive to ceftazidime, but resistant to amoxicillin/clavulanate. Overall, *Enterobacter* spp from goat faecal samples were sensitive to ceftazidime (86.4%) and trimethoprim/sulfamethoxazole (81.8%) but resistant to imipenem (27.3%) and meropenem (31.8%) (Table 4).

Table 1: Antibiotic susceptibility profile of *Enterobacter* spp isolated from streams in some rural areas in Akwa Ibom State, Nigeria

***Enterobacter* spp**	**No of isolate susceptibility to selected antibiotics (%)**									
	Status	**IPM**	**MEM**	**DOX**	**CAZ**	**CIP**	**AMC**	**FOX**	**S**	**SXT**
E. cloacae (n=15)	S	7 (43.8)	6 (40.0)	7 (43.8)	13 (86.7)	10 (66.7)	13 (86.7)	3 (20.0)	5 (33.3)	9 (60.0)
	I	3 (20.0)	5 (33.3)	2 (13.3)	0	5 (33.2)	2 (13.3)	4 (26.7)	2 (13.3)	2 (13.3)
	R	5 (33.3)	4 (26.7)	6 (40.0)	2 (13.3)	0	0	8 (53.3)	8 (53.3)	4 (26.7)
E. aerogenes (n=13)	S	7 (53.8)	8 (61.5)	10 (76.9)	11 (84.6)	7 (53.8)	12 (92.3)	5 (38.5)	7 (53.8)	11 (84.6)
	I	2 (15.4)	0	0	0	4 (30.7)	0	2 (15.4)	0	0
	R	4 (30.7)	5 (38.5)	3 (23.1)	2(15.4)	2 (15.4)	1 (7.7)	6 (46.1)	6 (46.1)	2 (15.4)
E. gergoviae (n=5)	S	3 (60.0)	2 (40.0)	3 (60.0)	5 (100.0)	3 (60.0)	4 (80.0)	5 (100.0)	4 (80.0)	4 (80.0)
	I	1 (20.0)	1 (20.0)	0	0	2 (40.0)	1 (20.0)	0	0	0
	R	1 (20.0)	2 (40.0)	2 (40.0)	0	0	0	0	1 (20.0)	1 (20.0)
E. cloacae subsp *dissolvens* (n=7)	S	6 (85.7)	6 (85.7)	5 (71.4)	7 (100.0)	4 (57.1)	5 (71.4)	3 (42.9)	4 (57.1)	4 (57.1)
	I	0	0	0	0	3 (42.9)	0	3 (42.9)	0	0
	R	1 (14.3)	1 (14.3)	2 (28.6)	0	0	2 (28.6)	1 (14.3)	3 (42.9)	3 (42.9)
E. mori (n=3)	S	0	0	2 (66.7)	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	3 (100.0)
	I	3 (100.0)	3 (100.0)	1 (33.3)	0	0	0	0	0	0
	R	0	0	0	0	0	0	1 (33.3)	1 (33.3)	0
Total (n=43)	S	23 (53.5)	22 (51.2)	27 (62.8)	39 (90.7)	27 (62.8)	32 (74.4)	18 (41.9)	22 (51.2)	31 (72.1)
	I	9 (20.9)	9 (20.9)	3 (7.0)	0	14 (32.6)	3 (7.0)	9 (20.9)	2 (4.7)	2 (4.7)
	R	11 (25.6)	12 (27.9)	13 (30.2)	4 (9.3)	2 (4.7)	3 (7.0)	16 (44.2)	19 (37.2)	10 (23.3)

S: Sensitive; I: Intermediate; R; Resistant; IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole; %: Percentage of the total isolates of each species of *Enterobacter* tested

Table 2: Antibiotic susceptibility profile of *Enterobacter* spp isolated from human faecal samples in some rural areas in Akwa Ibom State, Nigeria

***Enterobacter* spp**	**No of isolate susceptibility to selected antibiotics (%)**									
	Status	**IPM**	**MEM**	**DOX**	**CAZ**	**CIP**	**AMC**	**FOX**	**S**	**SXT**
E. aerogenes (n=18)	S	9 (50.0)	9 (50.0)	10 (55.6)	10 (55.6)	9 (50.0)	16 (88.9)	8 (44.4)	9 (50.0)	12 (66.7)
	I	7 (38.9)	4 (32.2)	1 (5.6)	0	8 (44.4)	0	3 (16.7)	0	0
	R	2 (11.1)	5 (27.8)	7 (38.9)	8 (44.4)	1 (5.6)	2 (11.1)	7 (38.9)	9 (50.0)	6 (33.3)
E. cloacae (n=15)	S	7 (46.7)	8 (53.3)	8 (53.3)	12 (80.0)	12 (80.0)	15 (100.0)	7 (46.7)	11 (73.3)	12 (80.0)
	I	4 (26.7)	1 (6.7)	1 (6.7)	0	3 (20.0)	0	2 (13.3)	1 (6.7)	0
	R	4 (26.7)	6 (40.0)	6 (40.0)	3 (20.0)	0	0	6 (40.0)	3 (20.0)	3 (20.0)
E. gergoviae (n=6)	S	2 (33.3)	1 (16.7)	2(33.3)	4 (66.7)	3 (50.0)	6 (100.0)	3 (50.0)	3 (50.0)	4 (66.7)
	I	3 (50.0)	4 (66.7)	0	0	2 (33.3)	0	0	0	0
	R	1 (16.7)	1 (16.7)	4 (66.7)	2 (33.2)	1 (16.7)	0	3 (50.0)	3 (50.0)	2 (33.3)
E. cloacae sub dissolvens (n=7)	S	3 (42.9)	4 (57.1)	4 (57.1)	6 (85.7)	3 (42.9)	7 (100.0)	5 (71.4)	5 (71.4)	7 (100.0)
	I	2 (28.6)	1 (14.3)	2 (28.6)	0	0	0	1 (14.3)	1 (14.3)	0
	R	2 (28.6)	2 (28.6)	1 (14.3)	1 (14.3)	4 (57.1)	0	1 (14.3)	1 (14.3)	0
Total (n=46)	S	21 (45.7)	22 (47.8)	24 (52.2)	32 (69.0)	27 (58.7)	44 (95.7)	23 (50.0)	28 (60.9)	35 (76.1)
	I	16 (34.8)	10 (21.7)	4 (8.7)	0 (14 (30.4)	13 (28.3)	0	6 (13.0)	2 (4.3)	0
	R	9 (19.6)	14 (30.4)	18 (39.1)		6 (13.0)	2 (4.3)	17 (37.0)	16 (34.8)	11 (23.9)

S: Sensitive; I: Intermediate; R; Resistant; IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole; %: Percentage of the total isolates of each species of *Enterobacter* tested

Table 3: Antibiotic susceptibility profile of *Enterobacter* spp isolated from dog faecal samples in some rural areas in Akwa Ibom State, Nigeria

***Enterobacter* spp**	**No of isolate susceptibility to selected antibiotics (%)**									
	Status	**IPM**	**MEM**	**DOX**	**CAZ**	**CIP**	**AMC**	**FOX**	**S**	**SXT**
E. cloacae (n=4)	S	2 (50.0)	2 (50.0)	3 (75.0)	3(75.0)	3 (75.0)	4 (100.0)	1 (25.0)	1 (25.0)	4 (100)
	I	0	0	1 (25.0)	0	0	0	2 (50.0)	1 (25.0)	0
	R	2 (50.0)	2 (50.0)	0	1 (25.0)	1 (25.0)	0	1 (25.0)	2 (50.0)	0
E. aerogenes (n=6)	S	2 (33.3)	3 (50.0)	5 (83.3)	6 (100.0)	3 (50.0)	6 (100.0)	4 (66.7)	3 (50.0)	4 (66.7)
	I	3 (50.0)	3 (50.0)	1 (16.7)	0	3 (50.0)	0	1 (16.7)	0	0
	R	1 (16.7)	0	0	0	0	0	1 (16.7)	3 (50.0)	2 (33.3)
E. hormaechei (n=1)	S	0	1 (100)	1 (100)	1 (100.0)	0	0	0	0	1 (100.0)
	I	0	0	0	0	1 (100.0)	0	0	0	0
	R	1 (100)	0	0	0	0	1 (100.0)	1 (100.0)	1(100.0)	0
E. quasiroggenkampii (n=2)	S	0	0	2 (100.0)	1 (100.0)	1 (100.0)	2 (100.0)	0	0	1 (50.0)
	I	1 (50.0)	1 (50.0)	0	0	0	0	1 (50.0)	0 2 (100)	0
	R	1 (50.0)	1 (50.0)	0	1 (100.0)	1 (100.0)	0	1 (50.0)		1 (50.0)
Total (n=13)	S	4 (30.8)	6 (46.2)	11 (84.6)	11 (84.6)	7 (53.8)	12 (92.3)	5 (38.5)	4 (30.8)	10 (76.9)
	I	4 (30.8)	4 (30.8)	2 (15.4)	0	4 (3.0.8)	0	4 (30.80	1 (7.7)	0
	R	5 (38.5)	3 (23.1)	0	2 (15.4)	2 (15.4)	1 (7.7)	4 (30.8)	8 (61.5)	3 (23.1)

S: Sensitive; I: Intermediate; R; Resistant; IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole; %: Percentage of the total isolates of each species of Enterobacter tested

Table 4: Antibiotic susceptibility of *Enterobacter* spp isolated from goat faecal samples in some rural areas in Akwa Ibom State, Nigeria

***Enterobacter* spp**	**No of isolate susceptibility to selected antibiotics (%)**									
	Status	**IPM**	**MEM**	**DOX**	**CAZ**	**CIP**	**AMC**	**FOX**	**S**	**SXT**
E. cloacae (n=5)	S	3 (60.0)	3 (60.0)	3 (60.0)	4 (80.0)	2 (40.0)	4 (80.0)	1 (20.0)	2 (40.0)	5 (100)
	I	1 (20.0)	1 (20.0)	1 (20.0)	0	3 (60.0)	0	2 (40.0)	2 (40.0)	0
	R	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	0	1 (20.0)	2 (40.0)	1 (20.0)	0
E. aerogenes (n=7)	S	5 (71.4)	6 (85.7)	6 (85.7)	6 (85.7)	2 (28.6)	7 (100.0)	6 (85.7)	5 (71.4)	5 (71.4)
	I	2 (28.6)	1 (14.3)	0	0	5 (71.4)	0	0	0	0
	R	0	0	1 (14.3)	1 (14.3)	0	0	1 (14.3)	2 (28.6)	2 (28.6)
E. cloacae subsp *dissolvens* (n=3)	S	2 (66.7)	1 (33.3)	3 (100.0)	3 (100.0)	2 (66.7)	3 (100.0)	1(33.3)	0	2 (66.7)
	I	0	0	0	0	1 (33.3)	0	0	1 (33.3)	1 (33.3)
	R	1 (33.3)	2 (66.7)	0	0	0	0)	2 (66.7)	2 (66.7)	0
E. quasiroggenkampii (n=2)	S	0	0	1 (50.0)	1 (50.0)	1 (50.0)	2 (100.0)	0	1 (50.0)	2 (100.0)
	I	0	0	0	0	1 (50.0)	0	0 2 (100)	0	0
	R	2 (100.0)	2 (100)	1 (50.0)	1(50.0)	0	0		1 (50.0)	0
E. bugandensis (n=3)	S	0	0	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	0	2 (66.7)	3 (100.0)
	I	1 (33.3)	2 (66.7)	0	0	0	0	2 (66.7)	0	0
	R	2 (66.7)	1 (33.3)	0	0	0	0	1 (33.3)	1 (33.3)	0
E. hormaechei (n=2)	S	0	0	1 (50.0)	2 (100.0)	1 (50.0)	0	1 (50.0)	1 (50.0)	1 (50.0)
	I	2 (100.0)	1 (50.0)	0	0	1 (50.0)	0	0	0	0
	R	0	1 (50.0)	1 (50.0)	0	0	2 (100.0)	1 (50.0)	1 (50.0)	1 (50.0)
Total (n=22)	S	10 (45.5)	10 (45.5)	17 (77.3)	19 (86.4)	11 (50.0)	19 (66.4)	9 (40.9)	11 (50.0)	18 (81.8)
	I	6 (27.3)	5 (22.7)	1 (4.5)	0	11 (50.0)	0	4 (18.2)	3 (13.6)	1 (4.5)
	R	6 (27.3)	7 (31.8)	4 (18.2)	3 (13.6)	0	3 (13.6)	9 (40.9)	8 (36.4)	3 (13.6)

S: Sensitive; I: Intermediate; R; Resistant; IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole; %: Percentage of the total isolates of each species of *Enterobacter* tested

Antibiotic susceptibility of *Enterobacter* spp to colistin

The minimum inhibitory concentration (MIC) of colistin for *Enterobacter* spp from water samples shows that 4 of 15 (26.7%) *E. cloacae* and 5 of the 13 (38.5%) *E. aerogenes* isolates were resistant to colistin. However, all 3 (100.0%) *E. mori* isolates were sensitive to colistin. Overall, 11 (25.6%) of the 43 *Enterobacter* spp from water samples were resistant to colistin (Table 5).

The MIC of colistin for *Enterobacter* spp from human faecal samples shows that 7 of 18 38.9%) *E. aerogenes,* 4 of 15 (26.7%) *E. cloacae* and 2 of 6 (33.3%) *E. gergoviae,* were resistant to colistin, while all the 7 (100%) *E. dissolvens* were sensitive to colistin. Overall, 28.3% of the 46 *Enterobacter* spp from human samples were resistant to colistin (Table 6).

From goat samples, 20.0% (n= 1/5) of *E cloacae*, 14.3% (n=1/7) of *E. aerogenes*, 33.3% (n=1/3) of *E cloacae* subsp *dissolvens*, 50.0% (n=1/2) *E. hormaechei*, and 100.0% of *E. quasiroggenkampii* were resistant to colistin, while all 3 (100.0%) *E. bugandensis* isolates were sensitive to colistin. Overall, 27.3% (n= 6/22) *Enterobacter* isolates from goat samples were resistant to colistin (Table 7).

Of the 13 *Enterobacter* spp isolated from dog faecal samples, 25.0% (n= 1/4) *E. cloacae*,16.7% (n=1/6) *E. aerogenes*, 100.0% (n=1/1) *E. hormaechei* and 100.0% (n=2/2) *E. quasiroggenkampii* isolates were resistant to colistin. Overall, 38.5% (n=5/13) *Enterobacter* isolates from dog samples were resistant to colistin (Table 8).

In summary, colistin resistance rate among all the *Enterobacter* isolates was 28.2% (n=35/124). The resistance rate among *Enterobacter* isolates from water samples (25.6%) was not significantly different from 28.3% from human, 27.3% from goat, and 38.5% from dog samples (x^2=0.8306, p=0.8421).

Multiple antibiotic resistance indices of *Enterobacter* spp

The MAR indices of *Enterobacter* spp. isolated from water samples showed that some *E. cloacae*, *E. aerogenes*, *E. aerogenes* and *E. cloacae* subspp *dissolvens* were resistant to similar antibiotic combinations such as cefoxitin and streptomycin, and imipenem, meropenem, streptomycin and trimethoprim/sulfamethoxazole. The MARI of all the 43 isolates from water samples ranged from 0.2 to 0.7, with resistance profiles ranging from 2 to 6 antibiotics (Table 9).

The MAR indices of *Enterobacter* spp. isolated for human faecal samples showed that *E. aerogenes*, *E. gergoviae*, and *E. cloacae* subsp *dissolvens* had MAR index of 0.2, while *E. cloacae* had MAR index of 0.4 and *E. gergoviae* MAR index of 0.7 (Table 10).

For goat faecal samples, MAR indices of *Enterobacter* spp showed that *E cloacae* subsp *dissolvens*, *E. bugandensis* and *E. hormaechei* had MAR index of 0.2, *E. aerogenes* had MAR index of 0.3, and *E. cloacae* had MAR index of 0.7 (Table 11).

The MAR indices of *Enterobacter* spp for dog faecal samples showed that *E. aerogenes* MARI index of 0.2, *E. quasiroggenkampii* and *E. aerogenes* MAR index of 0.3, and *E. quasiroggenkampii* had MAR index of 0.6 (Table 12).

Multidrug resistance profiles of *Enterobacter* spp

Of the total 124 *Enterobacter* isolates, 43 (34.7%) were MDR. As shown in Tables 9-12, 13 of 43 (30.2%) *Enterobacter* isolates from water samples are MDR, 19 of 46 (41.3%) from human samples were MDR, 6 of 22 (27.3%) from goat samples are MDR, and 5 of 13 (38.5%) from dog samples are MDR isolates. The difference in MDR rate with respect to sample source is not statistically significant (x^2=1.882, $\underline{p}$=0.5974).

***Mcr-1* gene carriage among colistin resistant *Enterobacter* spp**

The result of the colistin resistance gene amplification showed that one *Enterobacter* spp from Ikot Abasi LGA and one *Enterobacter* spp from Uruan LGA, both from human faecal samples, carried the mobile colistin resistance (*mcr-1*) gene, while *Enterobacter* spp from Mbo LGA isolated from goat faecal sample did not carry *mcr-1* colistin gene (Fig 1).

Table 5: Minimum inhibitory concentration (MIC) of Colistin for *Enterobacter* spp. isolated from water sample

***Enterobacter* isolate**	**No of isolate**	**Colistin MIC**	
		I ≤2µg/ml (%)	**R ≥4µg/ml (%)**
E. cloacae	15	11 (73.3)	4 (26.7)
E. aerogenes	13	8 (61.5)	5 (38.5)
E. gergoviae	5	4 (80.0)	1 (20.0)
E. dissolvens	7	6 (85.7)	1 (14.3)
E. mori	3	3 (100.0)	0
	43	**32 (74.4)**	**11 (25.6)**

I: Intermediate ≤2; R: Resistance ≥4; %: percent of the number of isolates for each species of *Enterobacter* tested

Table 6: Minimum inhibitory concentration (MIC) of colistin for *Enterobacter* spp. isolated from human faecal samples

***Enterobacter* isolate**	**No of isolate**	**Colistin MIC**	
		I (<2µg/ml) %	**R (≥4µg/ml) %**
E. aerogenes	18	11 (61.1)	7 (38.9)
E. cloacae	15	11 (73.3)	4 (26.7)
E. gergoviae	6	4 (66.7)	2 (33.3)
E. dissolvens	7	7 (100.0)	0
	46	**33 (71.3)**	**13 (28.3)**

I: Intermediate ≤2; R: Resistance ≥4; %: percent of the number of isolates for each species of *Enterobacter* tested

Table 7: Minimum inhibitory concentration (MIC) of colistin for *Enterobacter* spp isolated from goat faecal samples

***Enterobacter* isolate**	**No of isolate**	**Colistin MIC**	
		I (<2µg/ml) %	**R (≥4µg/ml) %**
E. cloacae	5	4 (80.0)	1 (20.0)
E. aerogenes	7	6 (85.7)	1 (14.3)
E. dissolvens	3	2 (66.7)	1 (33.3)
E. quasiroggenkampii	2	0	2 (100.0)
E. hormaechei	2	1 (50.0)	1 (50.0)
E. bugandensis	3	3 (100.0)	0
	22	**16 (72.7)**	**6 (27.3)**

I: Intermediate ≤2; R: Resistance ≥4; %: percent of the number of isolates for each species of *Enterobacter* tested

Table 8: Minimum inhibitory concentration (MIC) of colistin for *Enterobacter* spp. isolated from dog faecal samples

***Enterobacter* isolate**	**No of isolate**	**Colistin MIC**	
		I (<2µg/ml) (%)	**R (≥4µg/ml)**
E. cloacae	4	3 (75.0)	1 (25.0)
E. aerogenes	6	5 (83.3)	1 (16.7)
E. hormaechei	1	0	1 (100.0)
E. quasiroggenkampii	2	0	2 (100.0)
	13	**8 (61.5)**	**5 (38.5)**

I: Intermediate ≤2; R: Resistance ≥ 4; %: percent of the number of isolates for each species of *Enterobacter* Tested

Table 9: Multiple antibiotic resistance (MAR) index and resistance profiles of *Enterobacter* spp. from water sample in rural communities in Akwa Ibom State

MARI	No of antibiotics	Resistance profiles	No of isolates	*Enterobacter* species/codes
0.2	2	DOX, S	1	*E. cloacae*-wIK/03
0.2	2	S, FOX	4	*E. cloacae*-wI/04, *E. aerogenes*-wIK/06, *E aerogenes*-wI/09, *E. dissolvens*-wU/12
0.2	2	FOX, CIP	1	*E. aerogenes*-wI/14
0.2	2	IMP, MEM	1	*E. gergoviae*-wM/07
0.2	2	DOX, SXT	1	*E. dissolvens*-wO/12
0.2	2	FOX, DOX	1	*E. cloacae*-wM/24
0.3	3	MEM, S, SXT*	1	*E. dissolvens*-wM/20
0.3	3	IPM, MEM, CAZ	2	*E. cloacae*-wU/18, *E. aerogenes* -wIK/04
0.3	3	MEM, CAZ, SXT*	1	*E. aerogenes*-wU/08
0.3	3	DOX, AMC, SXT*	1	*E. dissolvens*-wI/01
0.3	3	MEM, SXT, DOX*	1	*E. cloacae*-wU/03
0.4	4	IPM, MEM, S, SXT*	3	*E. cloacae*-wU/02, *E. aerogenes*-wM/10, *E. dissolvens*-wIK/21
0.4	4	IPM, DOX, S, FOX*	3	*E. cloacae*-wU/24, *E. cloacae*-wI/02, *E. cloacae*-wO/10
0.6	5	MEM, CAZ, FOX, S, SXT*	1	*E. cloacae*-wO/06
0.6	5	IPM, MEM, DOX, S, FOX*	1	*E. cloacae*-wU/04
0.7	6	IPM, MEM, DOX, S, CIP, FOX*	1	*E. aerogenes*-wU/20

MAR index in this study was defined as the ratio of the number of antibiotics resisted by an isolate to the total number of antibiotics the isolate was exposed to (12b), while MDR in this study was defined as resistance of an isolate to at least one to three classes of antimicrobial agent tested (14) IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole (MAR INDEX= 34.9%) ; E- *Enterobacter*, w- water, I-Ikono, , O-Obot Akara, M- Mbo, U-Uruan, IK - Ikot Abasi; * = Multidrug Resistant (MDR)

Table 10: Multiple antibiotic resistance (MAR) index of *Enterobacter* spp. from human faecal samples in rural communities in Akwa Ibom State, Nigeria

MAR index	Number of antibiotics	Resistance profiles	No of isolates	*Enterobacter* species/codes
0.2	2	DOX, S	1	*E. aerogenes*-hM/07
0.2	2	CAZ, FOX	2	*E. aerogenes*-hM/26, *E. gergovia*e-hM/20
0.2	2	DOX, SXT	2	*E. cloacae*-hI/31, *E. gergovia*e-hU/06
0.2	2	IPM, MEM	3	*E. cloacae*-hM/30, *E. dissolvens*-hIb/10, *E. dissolvens* -hM/35
0.3	3	DOX, S, FOX*	3	*E. aerogenes*-hIk/11, *E. gergovia*e-hIk/26, *E. cloacae* -hM/28
0.3	3	DOX, S, SXT*	1	*E. cloacae*-hI/30
0.3	3	CAZ, AMC, FOX*	1	*E. aerogenes*-hu/33
0.3	3	IPM, MEM, DOX	1	*E. cloacae*-hO/09
0.3	3	MEM, DOX, FOX*	1	*E. cloacae*-hI/06
0.4	4	MEM, CAZ, S, FOX*	1	*E. cloacae*–hU/05
0.4	4	MEM, CAZ, S, SXT*	1	*E. aerogenes*–hO/14
0.4	4	DOX, CAZ, S, FOX*	1	*E. gergovia*e-hIK/26
0.4	4	MEM, DOX, S, FOX*	1	*E. aerogenes*–hU/17
0.4	4	MEM, CAZ, FOX, SXT*	1	*E. aerogenes*–hO/28
0.4	4	MEM, IPM, DOX, SXT*	1	*E. cloacae*-hU/19
0.5	5	DOX, CAZ, GP, FOX, Z*	1	*E. aerogenes*–hlk/03
0.5	5	IPM, MEM, CAZ, S, SXT*	1	*E. aerogenes*–hlb/01
0.5	5	DOX, CAZ, FOX, S, SXT*	2	*E. aerogenes*–hlb/36, *E aerogenes*-hO/34
0.5	5	IPM, MEM, DOX, S, SXT*	1	*E. aerogenes* hI /13
0.5	5	IPM, MEM, DOX, S, FOX*	1	*E. cloacae*–hU/31
0.7	6	IPM, MEM, DOX, CIP, S, SXT*	1	*E. gergovia*e–hl/26

MAR index in this study was defined as the ratio of the number of antibiotics resisted by an isolate to the total number of antibiotics the isolate was exposed to (14), while MDR in this study was defined as resistance of an isolate to at least one to three classes of antimicrobial agent tested (16). IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole (MARI = 60.9%) ; E-*Enterobacter*, h-human, I-Ikono, lb-Ibiono, O-Obot Akara, M- Mbo, U-Uruan, IK - Ikot Abasi; * = Multidrug Resistant (MDR)

Table 11: Multiple antibiotic resistance (MAR) index of *Enterobacter* spp. from goat faecal sample in rural communities in Akwa Ibom State

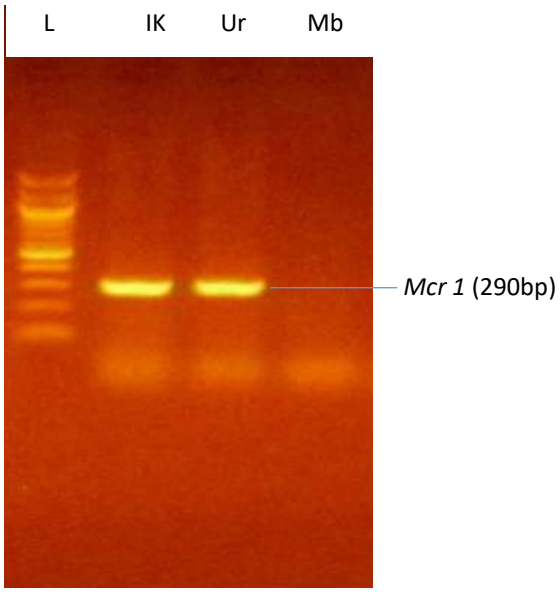
MAR index	No of antibiotics	Resistance profiles	No of isolates	Antibiotic class	Resistance categories	*Enterobacter* species /codes
0.2	2	FOX, S	1	2	-	*E. dissolvens*–gl/02
0.2	2	IPM, MEM	1	1	-	*E. bugandensis*–gM /22
0.2	2	DOX, FOX	1	2	-	*E. cloacae*–gIb/33
0.2	2	DOX, AMC	1	2	-	*E. bugandensis*–gIk/26
0.3	3	DOX, FOX, SXT*	1	3	MDR	*E. aerogenes*–glb/29
0.4	4	IPM, MEM, S, FOX*	2	3	MDR	*E. dissolvens*–gO/21, *E. gergoviae*–gM/03
0.6	5	MEM, AMC, FOX, S, SXT*	1	5	MDR	*E. bugandensis*-glk/06
0.6	5	IPM, MEM, DOX, CAZ, FOX*	1	4	MDR	*E. gergoviae*-gM/15
0.7	6	IPM, MEM, CAZ, AMC, S, FOX*	1	4	MDR	*E. cloacae*–gU/27

MDR in this study was defined as resistance of an isolate to at least one to three classes of antimicrobial agent tested (14), while MAR index in this study was defined as the ratio of the number of antibiotics resisted by an isolate to the total number of antibiotics the isolate was exposed to (16). IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole (MARI=27.3%); E- *Enterobacter*, g-goat, I-ikono, lb-lbiono, O-Obot Akara, M- Mbo, U-Uruan, Ik - Ikot Abasi; * = Multidrug Resistant

Table 12: Multiple antibiotic resistance (MAR) index of *Enterobacter* spp from dog faecal samples in rural communities in Akwa Ibom State

MAR index	Number of antibiotics	Resistance profiles	No of Isolates	Antibiotic class	Resistance categories	*Enterobacter* species /codes
0.2	2	IPM, SXT	1	2	-	*E. aerogenes*-dI/22
0.3	3	CIP, S, SXT*	1	3	MDR	*E. quasiroggenkampii*-dM/10
0.3	3	FOX, S, SXT*	1	3	MDR	*E. aerogenes*-dIb/34
0.3	3	IPM, MEM, CAZ	1	2	-	*E. cloacae*-dlk/01
0.4	4	IPM, MEM, S, FOX*	1	3	MDR	*E. cloacae*-dU/04
0.4	4	IPM, AMC, S, FOX*	1	4	MDR	*E. hormaechei*-dlk/29
0.6	5	IPM, MEM, CAZ, S, FOX*	1	4	MDR	*E. quasiroggenkampii* -dM/25

MDR in this study was defined as resistance of an isolate to at least one to three classes of antimicrobial agent tested (14), while MAR index in this study was defined as the ratio of the number of antibiotics resisted by an isolate to the total number of antibiotics the isolate was exposed to (16). IPM: Imipenem; MEM: Meropenem; DOX: Doxycycline; CAZ: Ceftazidime; CIP: Ciprofloxacin; AMC: Amoxicillin/Clavulanate; FOX: Cefoxitin; S: Streptomycin; SXT: Trimethoprim/Sulfamethoxazole (MARI=46.2%); E- *Enterobacter*, d- dog, I-ikono, lb-lbiono, M-Mbo, U-Uruan, IK - Ikot Abasi; * = Multidrug Resistant



L – DNA marker lane
Ik- Sample from Ikot Abasi Local Government Area
Ur – Sample from Uruan Local Government Area
Mb –Sample from Mbo Local Government Area

Fig 1: Agarose gel electrophoresis picture of *mcr-1* amplicon

Discussion:

Antibiotic resistance arises when bacteria mutate and reduce the efficiency of the antibiotics used to treat infections caused by them. This constitutes one of the most critical challenges affecting public health in the 21st century. Human-animal-environment interactions are critical for antimicrobial containment, according to the One Health concept (17,18). Antibiotic use in medical, veterinary care, and agriculture has contributed significantly to the evolution of bacterial resistance (18). This is true for Akwa Ibom State, Nigeria, where antibiotic resistance occurs across the study locations in water, humans, and animals.

The isolation of antibiotic-resistant *Enterobacter* species in water, human and animal sources is of public and clinical significance, as over-use of antibiotics may lead to treatment failure, extra length of hospital stays, increased mortality and economic losses (19). In this study, the antibiotic susceptibility profile of *Enterobacter* isolates from water, human, goat and dog samples analyzed showed varying outcomes. Most of the *Enterobacter* isolates from water were resistant to cefoxitin and streptomycin, which may be due to the wide availability of these antibiotics because they are cheap and therefore consistently used even by rural dwellers. This finding is consistent with the study of Tula et al., (20), which reported that majority of *Enterobacter* isolates were resistant to streptomycin. Previous studies in Abakaliki, Nigeria (21) and Cape Town, Ghana (22), have reported similar resistance rates.

Among the *Enterobacter* isolates, *E. cloacae* subspp *dissolvens* was resistant to trimethoprim/sulfamethoxazole. This may be due to the acquisition of resistant genes from the parent genus *E cloacae*. *Enterobacter aerogenes* and *E. cloacae* exhibited the highest rate of resistance to doxycycline, cefoxitin, streptomycin, and colistin, probably due to the frequent use of these antibiotics for treatment of related infections. High resistance to these antibiotics, especially colistin, by enteric pathogens including *Enterobacter* species in water sources has been previously reported (23).

The high prevalence of resistance by *Enterobacter* isolates to most of the commonly used antibiotics in this study constitutes a public health risk because these antibiotics may not be effective in treating infections that may occur in individuals who utilize the water sources for drinking or other domestic purposes. This study also recorded resistance to imipenem, meropenem, and ciprofloxacin among the *Enterobacter* isolates. This is reasonably expected since these antibiotics, particularly the carbapenems, are now commonly abused as they are available over-the-counter and dispensed without medical authorization. However, the resistance observed among the isolates might suggest the occurrence of cross-resistance, which occurs when an organism is resistant to an antibiotic to which it has not been exposed, probably because of acquisition of resistance markers to related antibiotics (24). Freshwater is a favorable environment for growth of many microorganisms such as the family Enterobacteriaceae (24), as a result of which they may serve not only as a vehicle for the spread of antibiotic-resistant organisms among humans and animals, but also as a means of resistance gene transfer in the natural bacterial ecosystems (25).

The antibiotic susceptibility profile of the *Enterobacter* isolates from human faeces indicates low resistance to trimethoprim/sulfamethoxazole due to infrequent use by members of the community and high resistance to imipenem due to over-the-counter availability, which is worrisome because carbapenems are regarded as antibiotics of last resort for treating Gram-negative bacterial infections (1,20). The clinical influence of carbapenemase producers has emerged worldwide due to their regular use in farm animal treatment (26). Medically valuable carbapenem-res-

istant *Enterobacter* species are a threat due to their resistance profile, accelerated emergence, and rapid transmission. Additionally, it can spread to other genera of Gram-negative organisms through genetic elements such as plasmids, insertion sequences, and transposons (27).

The emergence of antibiotic-resistant bacteria in animal populations may have important human public health consequences if isolates are transmitted to humans from their pets or via faecal contamination of water bodies (28). Understanding the prevalence of antibiotic resistance among *Enterobacter* isolates is thus important both from veterinary medicine and public health perspectives. Multidrug resistance is the most important challenge encountered in treating microbial infections, and there is an exponential increase in MDR over the last decade (28). The emergence of MDR *Enterobacter* spp has a negative impact on the clinical outcomes of infected patients with concomitant increase in morbidity and mortality (29).

This study found that *Enterobacter* spp*.* from goat faecal samples were sensitive to imipenem, ceftazidime and amoxicillin/clavulanate acid with very low resistance to trimethoprim/ sulfamethoxazole, probably due to lack of exposure to these antibiotics, as there are no veterinary services in the rural communities studied. This observation may be partly supported by the fact that *Enterobacter* isolates from human samples had the highest MDR rate (41.3%), compared to water and animal samples, although the difference in rates was not statistically significant (x^2=1.882, p=0.5974). However, overuse of antibiotics for treatment of infections in humans can create 'selection pressure' for emergence of MDR strains.

Enterobacter isolates from dog faecal samples in this study were 100% sensitive to amoxicillin/clavulanic acid, and 84.6% sensitive to doxycycline and ceftazidime. This high sensitivity rate may be due to lack of exposure to these antibiotics in the rural setting. However, there was a high rate of resistance to streptomycin, which may be due to overuse of streptomycin as Penstrep (penicillin and streptomycin) combination to treat bacterial infections in veterinary medicine (30), which they may acquire through transfer of resistance genes from the waste food that they eat while scavenging dump sites, as they are free-roaming animals in rural areas. Studies conducted by Mezzatesta *et al.* (31) reported that almost all *Enterobacter* isolates from faecal samples of goats and dogs exhibited resistance to amoxicillin/clavulanic acid, ceftazidime and ampicillin, possibly due to AmpC β-lactamase production, which strongly suggests dissemination of resistant isolates among the animals via direct spread from animal to animal, or indirect transmission from sources in the environment.

In the present study, minimum inhibitory concentrations (MICs) of colistin were determined for all *Enterobacter* isolates from water, human, goat and dog faecal samples. The overall colistin resistance rate among the *Enterobacter* isolates was 28.2%, and this rate was higher for isolates from dog samples (38.5%) than humans (28.3%), goats (27.3%) and water (25.6%) samples, although the difference in rates between sample sources was not statistically significant (x^2=0.8306, p=0.8421). Among the 43 *Enterobacter* isolates from water samples, 11 (25.6%) were resistant to colistin, and this could be as a result of transfer of colistin resistance by plasmid between different Gram-negative bacilli in the water body, which could lead to antibiotic resistance, especially in a community with low exposure to colistin.

Also, the rate of colistin resistance of *Enterobacter* isolates in human faecal samples was 28.3% (n=13) with *E. cloacae, E. aerogenes, E. gergoviae* and *E. dissolvens* being the implicated isolates. Resistance to colistin in this study may be attributed to the gradual use of colistin in farm animals like poultry and pigs which are reared by some members of the communities and are eventually consumed by the people who ordinarily do not take the antibiotics themselves (32). The *Enterobacter* isolates from the goat faecal samples were resistant to colistin (27.3%, n=6). This relatively high resistance rate may have occurred from consumption of colistin resistant *Enterobacter*-contaminated water, from the only source of drinking water for both man and animals in the community (33). The level of resistance of *Enterobacter* spp from dog faecal samples to colistin was 38.5% (n=5). This resistance rate could be attributed to the dogs coming in contact with human faecal wastes within the environment and their exposure to the streams, which may harbor colistin-resistant *Enterobacter* spp (33).

Although colistin was considered a drug of last resort for treatment of infections caused by multidrug-resistant organisms (MDROs), resistance to it is developing in recent times, probably due to its increased use in humans and continuous unauthorized use in farm animals such as poultry and pigs reared by some members of the communities. Colistin-resistant isolates may spread through the water and food chain to reach the populace who had not previously been exposed to colistin

(32,33). It is important to note that most antimicrobial-resistant non-clinical *Enterobacter* isolates can enter the food chain undetected (34). Previous reports indicate that these MDR bacteria can transfer their resistant genes from animals to humans through the use of glycopeptides growth promoters such as avoparcin in feed animals (27).

According to Thenmozhi et al., (35), MARI of >0.2 indicate resistant isolates are from environment where antibiotics are frequently used, while MARI of ≤0.2 indicates infrequent antibiotic usage. The MAR indices of *Enterobacter* spp isolated from water samples in our study showed *E. cloacae*, *E. aerogenes*, and *E. cloacae* subspp *dissolvens* exhibited resistance to two drug combinations probably due to low or no exposure antibiotics in the environment. Some isolates from Issiet Ekim in Uruan LGA, Isong Inyang in Mbo LGA, and Ikot Atan in Ikot Abasi LGA were resistant to three or more antibiotics, which implies that these isolates may carry antibiotic resistance genes probably acquired from other bacilli within the water source, brought about through human activities, runoff from erosion downstream, or agricultural activities in the farmlands around the vicinity of the stream.

Three *Enterobacter* spp from human faecal samples exhibited resistance to two drugs and had the MAR index of 0.2, which may indicate low exposure from infrequent antibiotic use. However, *E. cloacae*, *E. aerogenes*, *E. cloacae* subspp *dissolvens* and *E. gergoviae* isolates from human faecal samples were resistant to at least three different antibiotics with MAR index ranging from 0.3 to 0.7, indicating indiscriminate or frequent antibiotic usage. This observation is supported by the fact that the population is predominantly rural dwellers who rely solely on drugs purchased from untrained drug vendors because of lack of access to physicians in the rural settings.

In goat faecal samples, the MAR index revealed that *E. cloacae* subspp *dissolvens* from Ikono, *Enterobacter bugandensis* from Mbo, and *Enterobacter hormaechei* from Ikot Abasi, were resistant to two antibiotics each with a MARI of 0.2, which implies low exposure to antibiotics. *Enterobacter aerogenes* from Ibiono were resistant to three antibiotics, and *E. gergoviae* from Uruan setting was resistant to six antibiotics, which may suggest that goats have been exposed to water containing antibiotic-resistant strains of *Enterobacter* species.

The MAR index of *Enterobacter* spp in dog faecal samples showed that *E. aerogenes* had an MAR index of 0.2, indicating low or no antibiotic exposure in the dogs in Ikot Ntuen community of Ikono LGA. *Enterobacter quasiroggenkampii* in Mbo LGA were resistant to five antibiotics with MARI of 0.6, indicating that these *E. quasiroggenkampii* isolates in Isong Inyang, Mbo LGA may have possibly acquired resistance to many antibiotics through gene transfers within the environment or dogs feeding on faecal samples of humans harboring resistant bacteria. A previous study indicate that multidrug-resistant bacteria can transfer their resistant genes from animals to humans through glycopeptide growth promoters such as avoparcin (27).

The resistance profiles observed in human, water, and dog samples show a relatively close connection between some *Enterobacter* spp from these sources, which may indicate transmission from the environment (water) to human or animals. For instance, in Tables 9-12, one isolate (*E. cloacae* wU/04) from water sample had the same resistance profile (IPM/MEM /DOX/S/FOX) as one isolate (*E. cloacae* hU/31) from a human faecal sample. This may suggest transmission of the same *E. cloacae* to humans through contaminated water. Also, one isolate (*E. cloacae* wIK/ 03) from water sample had the same resistance profile (DOX/S) with one isolate (*E. aerogenes* hM/07) from human sample, while two isolates from goat samples (*E. dissolvens* gO/21 and *E. gergoviae* gM/03) had the same resistance profile (IPM/MEM /S/FOX) with one isolate (*E. cloacae* dU/ 04) from dog sample. Although these isolates shared the same resistance profile, they are different species of *Enterobacter*, which make them remotely unrelated, and therefore unlikely that there is transmission of these isolates from water to human or animal. Antibiotic resistance profile is known to be a poor discriminatory tool for epidemiological typing.

The high MAR indices obtained in this study, with resistance to many antibiotics including imipenem, meropenem, doxycycline, ciprofloxacin, ceftazidime, amoxicillin-clavulanic acid and colistin, is worrisome, as this may depict the existence of high-level antibiotic-resistant *Enterobacter* spp in the study area. The emergence of colistin resistance among *Enterobacter* isolates agrees with a previous study (36), due to increasing use for colistin in the treatment of carbapenem-resistant bacterial infections (36). Colistin has been regarded as the last-line therapeutic option against carbapenem-resistant Gram-negative bacteria (37). The detection of colistin-resistant (*mcr-1*) gene in some of the *Enterobacter* isolates in our study indicates a potential for exchange of genes at the human-animal-environment interface

among *Enterobacter* spp and other Gram-negative bacilli. Resistance to colistin may also be due to several mechanisms such as beta-lactamase production, efflux pumps, altered porin channels, and intrinsic low permeability of *Enterobacter* spp. These mechanisms may also contribute to the high level of resistance among the *Enterobacter* isolates, resulting in the high MDR phenotypes.

This study has some limitations. First, the study was conducted in only 6 selected rural communities in Akwa Ibom State, which make findings not generalizable to the entire population in the rural communities and urban areas of the State. However, based on the scientific method of sampling used to select the six communities, we believe there is a fair representation of the population in the State. Secondly, the study was limited by sample size variation across the sample sources; water (n=43), human (n=46), goat (n= 22), and dog (n=13), however comparisons were made using unique denominators for each sample source, which allowed accurate comparisons of prevalence of MDR and colistin resistance between the sample sources. Lastly, the study was restricted to molecular testing of only the *mcr-1* gene in colistin-resistant isolates, while other resistance mechanisms were not investigated. This could explain the wide disparity between phenotypic colistin resistance and genetic basis of this resistance. There is need to investigate further other molecular mechanisms responsible for colistin resistance in the study area.

Conclusion:

The findings of this study indicate that most *Enterobacter* isolates in the rural setting were resistant to cefoxitin and streptomycin but susceptible to ceftazidime, amoxicillin/clavulanic acid and trimethoprim/sulphamethoxazole. Multiple antibiotic resistance indices, multidrug resistance profiles and colistin resistance rates were generally high for the *Enterobacter* isolates from water, human, goat and dog faecal samples. An effective antimicrobial stewardship and surveillance system should be put in place to contain emergence and spread of multidrug and colistin-resistant *Enterobacter* spp in both human and animal populations in Akwa Ibom State, Nigeria.

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Contribution of authors:

AIT and UJU designed the study, performed laboratory analysis, wrote the original draft of the manuscript and reviewed the write-up. BM, UFZ and NEI were involved in sample collection and literature review. All authors critically reviewed the final manuscript.

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Original Article **Open Access**

Isolation and identification of heavy metal-tolerant bacteria from e-waste-contaminated soils and their potential for bioleaching of waste printed circuit boards

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Abstract:

Background: Informal handling and disposal of electronic waste can release heavy metals into soil and select for metal-tolerant microorganisms. This study aimed to isolate and identify heavy metal-tolerant bacteria from soils around two informal e-waste handling sites in Ilorin, Nigeria, assess changes in dissolved metal concentrations during 21 days of sequential enrichment with waste printed circuit boards (WPCBs), and evaluate the isolates as potential candidates for future bioleaching studies.
Methodology: In this study, samples of soils in two metal-polluted markets (Sango Kulende and Ipata Oloje Spare Parts) and a non-market control zone at Ilorin, Nigeria, were collected at 5 sub-locations in each site. Powdered waste printed circuit boards (WPCBs) were added to the soil samples and cultured in Luria Bertani (LB) broth at successively growing pulp densities (1-5 per cent) for 21 days. Aliquots of the cultures were diluted in 0.85% NaCl solution and plated on nutrient agar plates, which were then incubated for 48-96 hours at 30°C. Pure isolates were identified by morphological and biochemical characteristics. Atomic absorption spectrophotometry (AAS) was used to confirm the presence of iron (Fe), copper (Cu), nickel (Ni), and zinc (Zn) in the WPCBs before and after soil enrichment.
Results: Four morphologically different isolates were obtained on nutrient agar following serial dilution of enriched cultures and morphological and biochemical characterisation identified them as *Lactobacillus plantarum, Bacillus subtilis, Proteus vulgaris* and *Staphylococcus aureus*. Monitoring AAS of culture supernatants in three replication weeks demonstrated the steady rise of dissolved metal concentration mainly during the first and third weeks with a significant rise of Cu and Ni concentrations, suggesting a continuous mobilization of metals by the bacterial isolates. After week three, the concentration of Cu in Ipata soil + powdered e-waste (IS +PEW) cultures was 14.429ppm, and the concentration of Ni in Sango soil + general e-waste (SS +GEW) cultures was 6.699ppm. The isolates, especially those of *B. subtilis* and *P. vulgaris*, exhibited strong tolerance to copper and nickel, which increased with increased exposure.
Conclusion: These results suggest that soil bacteria used in informal e-waste recycling locations can be utilized as a bioleaching source and should be more explored regarding their resistance and scalability.

Keywords: Electronic waste; bioleaching; heavy metal tolerance; printed circuit boards; bioremediation

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Isolement et identification de bactéries tolérantes aux métaux lourds dans des sols contaminés par des déchets électroniques et leur potentiel de biolixiviation des cartes de circuits imprimés usagées

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Résumé:

Contexte: La manipulation et l'élimination informelles des déchets électroniques peuvent libérer des métaux lourds dans le sol et favoriser la sélection de micro-organismes tolérants aux métaux. Cette étude visait à isoler et identifier des bactéries tolérantes aux métaux lourds dans les sols de deux sites informels de traitement des déchets électroniques à Ilorin, au Nigéria, à évaluer les variations de concentration des métaux dissous au cours de 21 jours d'enrichissement séquentiel avec des déchets de circuits imprimés (DCI), et à évaluer les isolats comme candidats potentiels pour de futures études de biolixiviation.
Méthodologie: Des échantillons de sol ont été prélevés dans deux marchés pollués par les métaux (Pièces détachées Sango Kulende et Ipata Oloje) et dans une zone témoin non marchande à Ilorin, au Nigéria, à cinq sous-emplacements de chaque site. Des déchets de circuits imprimés (DCI) réduits en poudre ont été ajoutés aux échantillons de sol et mis en culture dans un bouillon Luria-Bertani (LB) à des densités de pulpe croissantes (1 à 5%) pendant 21 jours. Des aliquotes des cultures ont été diluées dans une solution de NaCl à 0,85% et étalées sur des plaques de gélose nutritive, qui ont ensuite été incubées pendant 48 à 96 heures à 30°C. Des isolats purs ont été identifiés par leurs caractéristiques morphologiques et biochimiques. La spectrophotométrie d'absorption atomique (SAA) a été utilisée pour confirmer la présence de fer (Fe), de cuivre (Cu), de nickel (Ni) et de zinc (Zn) dans les WPCB avant et après enrichissement du sol.
Résultats: Quatre isolats morphologiquement différents ont été obtenus sur gélose nutritive après dilutions en série de cultures enrichies. Leur caractérisation morphologique et biochimique a permis de les identifier comme *Lactobacillus plantarum, Bacillus subtilis, Proteus vulgaris* et *Staphylococcus aureus*. Le suivi par spectrophotométrie d'absorption atomique (SAA) des surnageants de culture pendant trois semaines de répétition a démontré une augmentation progressive de la concentration en métaux dissous, principalement au cours des première et troisièmes semaines, avec une hausse significative des concentrations de cuivre (Cu) et de nickel (Ni), suggérant une mobilisation continue des métaux par les isolats bactériens. Après trois semaines, la concentration de Cu dans les cultures de sol d'Ipata + déchets électroniques en poudre (IS+PEW) était de 14,429 ppm, et celle de Ni dans les cultures de sol de Sango + déchets électroniques généraux (SS+GEW) était de 6,699 ppm. Les isolats, en particulier ceux de *B. subtilis* et *P. vulgaris,* ont présenté une forte tolérance au cuivre et au nickel, qui augmentait avec l'exposition.
Conclusion: Ces résultats suggèrent que les bactéries du sol utilisées dans les sites informels de recyclage des déchets électroniques peuvent servir de source de biolixiviation et méritent d'être étudiées plus en détail quant à leur résistance et leur potentiel d'application à grande échelle.

Mots-clés: Déchets électroniques; biolixiviation; tolérance aux métaux lourds; circuits imprimés; bioremédiation

Introduction:

Electronic waste (e-waste) is among the fastest growing waste in the world as its volume is affected by the shortening of product life cycles, the rapid pace at which technology takes over, and the rise in electronic device consumption per person (1). Existing estimates show that annual generation of e-waste in the world amounts to over 53 million metric tons of e-waste and hardly a small proportion of this e-waste is collected and recycled in a responsible manner (1).

Waste printed circuit boards (WPCBs) are the most problematic and complex items of e-waste. They are a heterogeneous mixture of polymers, ceramics, and metals including economically valuable copper, gold, and palladium and environmentally hazardous nickel, lead, and cadmium (2,3). Poor disposal through open burning, acid leaching, or landfill at informal recycling sites in the low and middle-income countries pollute the surrounding soils and water masses, with ripple effects on the microbial population, plant growth, and human health (4).

The traditional methods of metal recovery of e-waste are based on pyrometallurgical smelting or hydro-metallurgical digestion in acid. The two systems are energy-easy, create secondary pollutants, and need lots of capital, and they are ill adapted to decentralised, low-resource environments (3). The biological solubilization of metals by microorganisms and their metabolites is called bioleaching and is a more sustainable alternative. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* chemolithotrophic acidophiles are the best-investigated bioleaching agents, but heterotrophic bacteria have been identified as viable alternatives, especially to alkaline or circumneutral-mixed waste matrices (5).

The repertoire of overlapping strategies used by microorganisms to tolerate heavy metals includes active efflux by active transport using RND, MFS, and P-type ATPase transporters, intracellular sequestration by metallothioneins, metal-binding peptides, enzymatic metal ion reduction and precipitation and extracellular chelation with biopolymers and siderophores (6,7). An example of this cation diffusion facilitator is CzcD, and the expression of this protein renders *Bacillus subtilis* resistant to Ni^{2+}, Cu^{2+}, Zn^{2+}, and Co^{2+}, and poly-γ-glutamic acid (γ-PGA) secretion denotes an extra cellular protection against zinc and copper toxicity (8). The cell

wall and exopolysaccharide matrix are the primary metal-binding surfaces in the lactic acid bacteria Lactobacillus plantarum, which facilitate passive biosorption of divalent cations in aqueous solution (9).

Informal electronics recycling in Nigeria, which operates centrally in urban markets in scrap yards, disposes of large amounts of e-waste on soils without containment (16). The soil surrounding these locations are unexploited source of native metal-tolerant bacteria that have evolved under long-term exposure to polymetallic contamination. Analyses of analogous West African samples have also recorded high levels of Fe, Cu, Ni and Zn in market-area soils but has received little systematic microbiological characterization of the endangering metallotolerant communities (4,16).

This research aimed to isolate and identify heavy metal-tolerant bacteria of the soils surrounding two informal e-waste handling sites in Ilorin, Kwara State, Nigeria, to determine their profiles of metal tolerance over a 21-day sequential enrichment phase using the WPCBs as their metal substrate, and to determine their suitability as candidates in subsequent bioleaching studies.

Materials and method:

Study area and sample collection:

The soil samples were taken at Sango Kulende Market and Ipata Oloje Spare Parts Market, Ilorin, Kwara State, Nigeria (around 8.5°N, 4.5°E). Both locations are described as having a high turnover of the trade in motor vehicle parts and second-hand electronic products and initial reconnaissance verified that there were observable metal littering, battery acid spills, and wiring waste lying on the soil surface. A third sample (control) was also collected directly in an adjacent non-market zone where no observed e-waste activity was observed.

Surface soil (depth 020 cm) at each site were sampled at 5 sub-locations with a sterile hand trowel, composite, and transferred in sterile and capped polypropylene containers. Products waste printed circuit boards (WPCBs) were obtained at an informal e-waste dismantling yard in the Challenge area of Ilorin.

Preparation of waste printed circuit boards:

The WPCBs were washed with distilled water, sheared, and ground to fine powder. PCB was immersed in a 10 M solution of NaOH (48 hours) to eliminate surface chemical coatings and washed using a running tap (to remove residual solder). The treated material was dried in 60°C and sieved to obtain two size fractions of material: powdered (≤120 μm) and granulated (≤250 μm) (10).

Metal analysis of WPCBs by atomic absorption spectrophotometry (AAS):

A 1-gm finely ground WPCB sample was agitated in 48 h aqua regia ($HCl:HNO_3$, 3:1 v/v) at room temperature The mixture was centrifuged at 5000xg/min and the product was filtered using Whatman No. 1 filter paper. Flame atomic absorption spectrophotometry (AAS) was used to determine the concentrations of iron (Fe), copper (Cu), nickel (Ni), and zinc (Zn). Measurements were conducted on untreated, NaOH-treated powdered and NaOH-treated granulated fractions (17).

Bacterial culture media preparation:

Nutrient agar (NA), prepared at 28 g L^{-1} as per the specifications of its manufacturer, was inoculated in conical flasks, plugged with cotton wool and autoclaved at 121° C. Luria-Bertani (LB) broth was prepared using peptone (10g L^{-1}), yeast extract (5g L^{-1}) and NaCl (10g L^{-1}), and again autoclaved at 121°C.

Sequential enrichment and bacterial isolation:

Sequential enrichment followed variation of an outlined procedure described by Kumar et al., (11). One gram of composite soil of each location was inoculated in 250 mL of Erlenmeyer flasks containing 100 mL of LB broth with the addition of 1% of WPCBs (particle size ≤150 m). The flasks were incubated at 30°C during week 1 at 150 rpm for 7 days. Then, 5 mL of enriched culture was inoculated into fresh LB with 2.5% WPCBs (week 2), and then with 5% WPCBs (week 3) and incubated further in the fresh LB. After 21 days, 5 mL aliquots were diluted in sequence (10^{-1} to 10^{-7}) in 0.85% NaCl solution and plated on NA. The plates were incubated for 48-96 hours at 30°C. Pure isolates were obtained by morphological selection and subsequent sub-culture in NA.

AAS monitoring of metal dissolution during enrichment:

Cultures were filtered with Whatman No. 1 paper at the conclusion of every enrichment cycle per week and centrifuged at 7,000x g, for 10 min. The cell-free supernatant was refiltered using 0.45 μm membrane filters to eliminate residual particulates and metal concentrations (Fe, Cu, Ni, Zn) in each supernatant were determined using the AAS and gave an indirect indication of the metal solubilization activity of the cell-free supernatant over a period.

Morphological and biochemical identification of bacterial isolates:

Colony morphology was documented in every isolate on NA (shape, margin, elevation, surface texture and pigmentation). Gram staining was done by performing the standard four-step procedure (12). Microscopy of the cell shape and arrangement was done under oil-immersion. Biochemical identification tests were performed with motility (triple sugar iron agar stab), catalase (3% H_2O_2), oxidase (Kovacs reagent on filter paper), coagulase (plasma slide test), indole (Kovacs reagent in tryptophan broth after 24 hours at 37°C), incubation with citric acid (citrate utilization agar), urease (plasma slide test), and gelatin agar flooded with mercuric chloride (gelatin hydrolysis). Comparison with standard keys was used to identify the isolates through biochemical enhancement of types (12).

Results:

Metal composition of WPCBs before and after NaOH treatment:

The analysis of the AAS showed that the top oil was the dominant Fe in untreated WPCBs (5.786 ppm), then Zn (2.775 ppm), Ni (1.549 ppm), and Cu (1.442 ppm) (Table 1). NaOH treatment significantly decreased the amount of Fe in the powdered (1.616 ppm) and granulated (1.429 ppm) fractions, which probably indicates the reagent washes away loosely bound impurity iron oxide phases.

On the contrary, it resulted in higher levels of Ni concentration following treatment in both fractions (powder: 2.063 ppm; granulated: 2.539 ppm), which implies that the alkaline wash revealed the hidden or blocked surfaces with nickel. The concentrations of Zn and Cu were relatively constant regardless of treatment.

Bacterial isolation and identification:

After 21 days of sequential enrichment in NA plates, 5 morphologically distinct types of colonies were obtained. The morphological and biochemical identification revealed four bacterial species; *Lactobacillus plantarum* from SS+PEW samples, *Bacillus subtilis* from IS+PEW and CS+GEW samples, *Proteus vulgaris* from IS+GEW sample, and *Staphylococcus aureus* from SS+GEW sample (Table 2). *Bacillus subtilis* was obtained in the two independent sample combinations suggesting that the bacteria were uniformly enriched when subjected to varied combinations of sites and substrates.

Table 3 shows the morphological and biochemical profiles used to identify the bacterial species. *Staphylococcus aureus* was identified as Gram-positive, coagulase-positive, urease-positive, and β-haemolytic on blood agar. *Bacillus subtilis* was identified as Gram-positive, endospore forming, oxidase-positive, and growth on starch hydrolysate. *Proteus vulgaris* was identified as Gram-negative, motile, indole-positive, Voges-Proskauer and urease-positive. *Lactobacillus plantarum* was identified as Gram-positive, non-motile, catalase-negative, with most testing negative for degradation, in line with the characteristics of homofermentative lactic acid bacteria.

Table 1: AAS metal concentrations (ppm) in WPCBs before and after NaOH treatment

S/N	Metal	Untreated (ppm)	Treated Powder (ppm)	Treated Granulated (ppm)
1	Iron (Fe)	5.786	1.616	1.429
2	Copper (Cu)	1.442	1.481	1.511
3	Nickel (Ni)	1.549	2.063	2.539
4	Zinc (Zn)	2.775	1.673	2.159

Table 2: Bacterial isolates recovered from sequential enrichment cultures after 21 days

S/N	Sample	Identified bacteria
1	Sango soil + Powdered e-waste (SS+PEW)	*Lactobacillus plantarum*
2	Ipata soil + Powdered e-waste (IS+PEW)	*Bacillus subtilis*
3	Ipata soil + Granulated e-waste (IS+GEW)	*Proteus vulgaris*
4	Sango soil + Granulated e-waste (SS+GEW)	*Staphylococcus aureus*
5	Control soil + Granulated e-waste (CS+GEW)	*Bacillus subtilis*

Table 3: Morphological and biochemical characteristics of the four bacterial isolates

Test	***S. aureus***	***B. subtilis***	***P. vulgaris***	***L. plantarum***
Shape	Cocci	Rod	Rod	Rod
Gram reaction	+	+	−	+
Motility	−	+	+	−
Endospore	+	+	−	−
Catalase	+	+	+	−
Oxidase	−	+	−	−
Coagulase	+	−	−	−
Indole	−	−	+	−
Citrate utilization	+	+	+	−
Urease	+	−	+	−
Gelatin hydrolysis	+	+	−	−
Methyl Red (MR)	+	+	+	+
Voges–Proskauer (VP)	−	−	+	−
Glucose fermentation	+	+	−	+
Sucrose fermentation	+	+	+	+
Lactose fermentation	+	+	−	+
Starch hydrolysis	−	+	−	+
H_2S production	−	+	+	−
Gas production	−	−	+	−
Haemolysis	+	−	+	−

+, positive; −, negative; FA, facultative anaerobe

Metal dissolution during sequential enrichment: The concentration of dissolved metal in culture supernatants varied significantly in three weeks of enrichment period. In week 1, the only significant metal mobilized was Ni and the anti-maxima values were obtained in the SS+PEW (mean 3.148±0.007 ppm) and IS+ GEW (mean 2.390±0.007 ppm) cultures (Table 3). Fe registered the highest in IS+PEW (mean 3.055 ± 0.008 ppm) whereas Zn and Cu were relatively low in all the samples.

At week 2, there was a sharp rise in copper concentrations in IS+PEW (mean 8.489 ±0.004 ppm), as well as IS+GEW (mean 7.941 ±0.007 ppm), SS+GEW (mean 7.871±0.006 ppm), and CS+GEW (mean 7.556±0.006 ppm), suggesting active mobilization of copper in the second enrichment passage at WPCBs concentration of 2.5. Ni still increased in IS+GEW (mean 4.525±0.003 ppm) and SS+GEW (mean 4.528±0.008 ppm) cultures (Table 4).

Table 3: AAS mean concentrations (ppm) of metals in culture supernatants after week 1 (day 7)

S/N	**Sample**	**Fe (ppm)**	**Cu (ppm)**	**Ni (ppm)**	**Zn (ppm)**
1	SS+PEW	1.006 ± 0.002	1.404 ± 0.003	3.148 ± 0.007	1.325 ± 0.003
2	IS+PEW	3.055 ± 0.008	2.542 ± 0.006	0.758 ± 0.003	1.812 ± 0.007
3	IS+GEW	1.712 ± 0.007	1.465 ± 0.003	2.390 ± 0.007	1.850 ± 0.004
4	SS+GEW	1.790 ± 0.010	1.462 ± 0.002	2.357 ± 0.004	1.848 ± 0.006
5	CS+GEW	1.488 ± 0.110	1.451 ± 0.008	0.994 ± 0.006	0.272 ± 0.002

Values are presented as mean ± standard deviation of triplicate determinations ($n = 3$)
SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

Table 4: AAS mean concentrations (ppm) of metals in culture supernatants after week 2 (day 14)

S/N	Sample	Fe (ppm)	Cu (ppm)	Ni (ppm)	Zn (ppm)
1	SS+PEW	1.225 ± 0.002	0.927 ± 0.009	3.403 ± 0.005	1.616 ± 0.006
2	IS+PEW	2.347 ± 0.006	8.489 ± 0.004	3.302 ± 0.006	1.952 ± 0.003
3	IS+GEW	1.614 ± 0.009	7.941 ± 0.007	4.525 ± 0.003	1.987 ± 0.008
4	SS+GEW	1.541 ± 0.011	7.871 ± 0.006	4.528 ± 0.008	1.987 ± 0.004
5	CS+GEW	1.749 ± 0.140	7.556 ± 0.006	2.993 ± 0.004	1.374 ± 0.006

Values are presented as mean ± standard deviation of triplicate determinations ($n = 3$)
SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

Table 5: AAS metal concentrations (ppm) in culture supernatants after week 3 (day 21)

S/N	Sample	Fe (ppm)	Cu (ppm)	Ni (ppm)	Zn (ppm)
1	SS+PEW	1.443 ± 0.004	0.449 ± 0.002	3.658 ± 0.008	1.906 ± 0.004
2	IS+PEW	1.638 ± 0.003	14.429 ± 0.008	5.846 ± 0.004	2.092 ± 0.006
3	IS+GEW	1.516 ± 0.004	14.416 ± 0.005	6.659 ± 0.006	2.123 ± 0.003
4	SS+GEW	1.292 ± 0.120	14.279 ± 0.004	6.699 ± 0.003	2.126 ± 0.007
5	CS+GEW	1.505 ± 0.043	13.661 ± 0.004	4.992 ± 0.007	2.476 ± 0.004

Values are presented as mean ± standard deviation of triplicate determinations ($n = 3$)
SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

The week 3 outcomes demonstrated an additional increase in dissolved Cu that reached its highest in the IS+PEW (mean 14.429±0.008 ppm), IS+GEW (14.416±0.005 ppm), SS+GEW (14.279±0.004 ppm), and CS+GEW (13.661± 0.004 ppm) cultures to the tune of about 10 times the result of week 1. Ni levels also increased up to mean values of 6.699±0.003 ppm in SS+GEW and 6.659±0.006 ppm in IS+GEW (Table 5). However, in contrast, week 1 showed higher Fe concentrations compared to week 3 in all the samples, which could suggest the precipitation of Fe or biosorption onto bacterial biomass.

Discussion:

The abundance of Fe in untreated WPCBs (5.786 ppm) and the relatively low level of Cu (1.442 ppm) here is in contrast with high-copper profiles previously reported in newer generation consumer electronics PCBs, which can contain Cu up to or above 20% by weight (2). The difference is probably due to the variation in age and kind of electronic equipment obtained in informal markets in Ilorin and the rather small sample sizes on which the digestion process in this paper was carried out. However, the four metal in the analytes Fe, Zn, Ni, and Cu are the most tenacious ones and microbiologically significant metals in the WPCB leachates, and the bacterial mobilization is extensively reported (5,13).

The significant, linear increase in dissolved Cu in all of the enrichment cultures between Week 1 and Week 3 is also worth noting and the approximate 10-fold rise in solubility of Cu between days 7 and 21 may indicate that the bacterial populations were experiencing adaptive enrichment as the WPCB pulp density increased (13). The trend resembles those shown in acidophilic cultures that oxidize iron, where serial sub-culturing in progressively higher PCB concentrations enhanced metal tolerance and have an accelerated rate of metal dissolution (13). The ability in the current research was demonstrated by heterotrophic mesophilic genera, as opposed to acidophilic chemolithotrophs that are widely reported in bioleaching literature, which expands the array of organisms with viable application to e-waste (13).

The most frequent isolation used in this study was *B. subtilis*, as it was isolated in two separate enrichment combinations. Cation diffusion facilitator CzcD supports Ni^{2+}, Cu^{2+}, and Zn^{2+} resistance in this species concurrently and poly-gamma-glutamic acid (g-PGA) secretion supplies further extracellular chelation of these

metals in excess conditions (8). At the same time, *B. subtilis* implements biosorption using cell wall peptidoglycans, extracellular polymeric substances (EPS) and bioprecipitation as supplementary mechanisms of coping with polymetallic stress which together explain its dominion in metal-contaminated habitats (7).

In weeks 2 and 3, a significant level of tolerance to Cu and Ni was observed (Cu: 14. 416 ppm; Ni: 6.659 ppm in IS+GEW). High-metal exposure of this Gram-negative organism triggers broad-spectrum stress response mechanisms and induces metal-resistance genes (5). Although *P. vulgaris* has not received as much attention on bioremediation as *Bacillus* or *Pseudomonas* species in bioremediation studies, its survival in this case under two independent soil enrichments with granulated WPCBs necessitates additional characterization of its tolerance machinery at a molecular level (5).

The fact that *L. plantarum* can survive in enrichment cultures is especially interesting, as lactic acid bacteria are not traditionally connected with metallic-polluted soil and the biosorption of copper through wine-relevant lactobacilli through cell wall binding mechanisms has been documented and more recent studies have verified the ability of *L. plantarum* to remove Zn and Cu in aqueous solution at up to 84% (9). *L. plantarum* provides extensive carboxyl, hydroxyl and phosphate groups on the cell wall-associated polar head of polysaccharides and protein to coordinate metal, thus allowing passive metal sequestration without spending metabolic energy which renders *L. plantarum* an object of biosorption-based metal capture as opposed to oxidative bioleaching itself (18).

In weeks 2 and 3, *S. aureus* exhibited tolerance of Cu and Ni. *S. aureus* metalliform resistance genes such as copper efflux operons (*copA/B*) and *czr* operon that regulates Zn/Co efflux, oftentimes overlap genomic loci that includes antibiotic resistance determinants on mobile genetic elements (MGEs) (14). Although the clinical importance of the organism provokes biosafety concerns when applied to industry, the fact that it grows in soils with a high concentration of metals supports the evidence that environmental *S. aureus* clonal forms that live in polluted niches develop metal resistance adaptations over time (14).

It is worth noting the observed decrease in Fe concentrations between Week 1 and Week 3 in all the enrichment cultures. In contrast to Cu and Ni, the dissolved Fe did not rise after two consecutive passages of enrichment though Fe was the most available metal within the original WPCB matrix. This trend is in line with microbiologically enhanced Fe^{2+} oxidation and subsequent circumneutral pH precipitation as iron oxyhydroxides, observed in heterotrophic biolagging systems (13). Alternatively, the reduction might be due to the sorption of Fe to bacterial biomass and EPS which are more heavily concentrated in mature cultures. Both interpretations are in line with the established tendency of the Bacillus to generate siderophores and EPS, which are highly coordinating in Fe^{3+} (7).

There are a few limitations to this study that should be considered. The identification of the bacteria was based only on phenotypic biomarkers; 16S rRNA gene sequencing a technique often considered the gold-standard of bacterial identification in the environment would give a more certain species-level name and demonstrate intraspecific variation in the *B. subtilis* population (19). Data in the AAS indicates total dissolved metal in bulk supernatants and makes no differentiation between biologically mobilized metals and metals liberated by abiotic dissolution. A more stringent evaluation of biological contribution would have been made possible by inclusion of abiotic WPCB-LB controls that lacked bacterial inoculation. In addition, the WPCB concentration employed in this case (≤5%) is small compared with industrial-related densities of pulp, say 10-20%, and tolerance of the isolation to the increased metal loads must be determined (20).

Conclusion:

This study showed that the soils surrounding informal recycling e-waste sites in Ilorin, Nigeria, contain a culture of great metal tolerant bacteria, which can live and develop in complex poly-metallic WPCB structures. The most common tolerant species were identified as *B. subtilis, P. vulgaris, S. aureus* and *L. plantarum,* and mobilization of Cu and Ni increased significantly in three consecutive enrichment passages.

The progressive enrichment method used was effective in the selection of metal-adapted populations that have potential bioleaching and biosorption activity. The future research must integrate 16S rRNA gene sequencing to identify assured strain, abiotic dissolution in controlled conditions to stringently ascribe the metal solubilization to microbial activity, and molecular investigation of efflux gene complementation and the expression of metal-binding proteins. These measures are preliminaries to the assessment of scalability of Nigerian indigenous e-waste-tolerant bacteria as those suitable to be used as sustainable metal recovery agents.

Contributions of authors:

AAS conceived and designed the study, supervised the research, and contributed to data interpretation; BTT conducted the laboratory experiments, collected and analysed the data, and prepared the initial manuscript draft; BMA contributed to the methodology, data interpretation, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript

Conflict of interests:

Authors declare no conflicts of interest.

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Artificial Intelligence (AI) use disclosure:

Authors declare that no generative AI tools were used in the original preparation, analysis, or writing of this manuscript. All experimental procedures, data analyses, interpretations, and written content were independently produced and verified by the authors.

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Original Article **Open Access**

Exploring *in-silico* antimicrobial potential of *Cymbopogon citratus* phytochemicals in the treatment of drug-resistant *Escherichia coli* using molecular docking and ADMET screening

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Abstract:

Background: Antimicrobial resistance (AMR) poses a serious global health threat, particularly by to multidrug-resistant *Escherichia coli*, which reduces the efficacy of conventional antibiotics. Medicinal plants such as *Cymbopogon citratus* have gained attention as potential alternative antimicrobial sources. This study evaluated the *in-silico* antimicrobial potential of *C. citratus* phytochemicals against drug-resistant *E. coli.*
Methodology: The chemical structures of bioactive compounds of *C. citratus* were identified from the literature and retrieved from PubChem. Molecular docking was conducted using AutoDock Vina against three E. coli protein targets (1JL2, 8EY4, and 8XZU). QSAR modeling was performed using the genetic function approximation method. Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties were predicted using ADMETlab 3.0 to assess pharmacokinetics and toxicity.
Results: Ligand 16,17-dimethoxyviolanthrone of *C. citratus* demonstrated the strongest binding affinity, particularly against 8XZU (−9.7 kcal/mol) *E. coli* protein, indicating stable protein–ligand interactions. However, ADMET analysis revealed high lipophilicity, poor solubility, strong plasma protein binding, CYP enzyme inhibition potential, and predicted hepatotoxicity and cardiotoxicity risks.
Conclusion: *Cymbopogon citratus* contains promising bioactive compounds with potential anti-E. coli activity, but structural optimization and experimental validation are necessary to address pharmacokinetic and safety concerns before therapeutic application.

Keywords: Antimicrobial resistance, *Cymbopogon citratus*, drug-resistant *Escherichia coli*, molecular docking, ADMET screening, QSAR modeling.

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Exploration *in silico* du potentiel antimicrobien des composés phyto-chimiques de *Cymbopogon citratus* dans le traitement d'*Escherichia coli* résistant aux médicaments par amarrage moléculaire et criblage ADMET

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Résumé:

Contexte: La résistance aux antimicrobiens (RAM) constitue une grave menace pour la santé mondiale, notamment en raison de l'émergence d'*Escherichia coli* multirésistant, qui réduit l'efficacité des antibiotiques conventionnels. Les plantes médicinales telles que *Cymbopogon citratus* suscitent un intérêt croissant en tant que sources antimicrobiennes alternatives potentielles. Cette étude a évalué *in silico* le potentiel antimicrobien des composés phytochimiques de *C. citratus* contre *E. coli* résistants aux antibiotiques.
Méthodologie: Les structures chimiques des composés bioactifs de *C. citratus* ont été identifiées dans la littérature et extraites de PubChem. Un amarrage moléculaire a été réalisé à l'aide d'AutoDock Vina contre trois protéines cibles d'*E. coli* (1JL2, 8EY4 et 8XZU). Une modélisation QSAR a été effectuée par la méthode d'approximation de la fonction génétique. Les propriétés d'absorption, de distribution, de métabolisme, d'excrétion

et de toxicité (ADMET) ont été prédites à l'aide d'ADMETlab 3.0 afin d'évaluer la pharmacocinétique et la toxicité. **Résultats:** Le ligand 16,17-diméthoxyviolanthrone de *C. citratus* a démontré la plus forte affinité de liaison, en particulier contre la protéine 8XZU d'*E. coli* (−9,7 kcal/mol), indiquant des interactions protéine-ligand stables. Cependant, l'analyse ADMET a révélé une lipophilie élevée, une faible solubilité, une forte liaison aux protéines plasmatiques, un potentiel d'inhibition des enzymes CYP et des risques prédits d'hépatotoxicité et de cardiotoxicité. **Conclusion:** *Cymbopogon citratus* contient des composés bioactifs prometteurs présentant une activité anti-*E. coli* potentielle, mais une optimisation structurale et une validation expérimentale sont nécessaires pour répondre aux préoccupations pharmacocinétiques et de sécurité avant toute application thérapeutique.

Mots-clés: Résistance aux antimicrobiens, *Cymbopogon citratus*, *Escherichia coli* résistante aux médicaments, amarrage moléculaire, criblage ADMET, modélisation QSAR.

Introduction:

According to the World Health Organi-, zation (WHO), antimicrobial resistance (AMR) is a global concern for all countries because it has a high cost for both health systems and national economies overall. The increase of AMR is caused by human activities, misuse, overuse, and incomplete use of antibiotics to treat, prevent, or control microbial infections in humans, animals, and plants (1). The WHO has emphasized the urgency of this crisis by identifying several priority pathogens that exhibit resistance to multiple antibiotic classes (2). These include *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa,* all of which are linked to high morbidity and mortality rates. Their ability to withstand conventional therapies underscores the pressing need for alternative and complementary antimicrobial agents capable of targeting MDR strains effectively.

Escherichia coli, a common cause of urinary tract infection mostly in females, has developed resistance to one of the commonly used antibiotics (fluoroquinolone). Patients who have infections caused by drug-resistant bacteria are at a very high risk of worse clinical outcomes or possibly death. Treatment of drug-resistant bacteria lead to increased healthcare cost (1). Worldwide, multidrug-resistant *E. coli* complicate treatment outcomes, and the burden is especially severe in low-and-middle-income countries, including Nigeria, where inappropriate antibiotic use and weak infection control practices contribute to high resistance rates. Reports from Nigeria highlight widespread resistance of bacteria to β-lactams, fluoroquinolones, and cephalosporins, further aggravating the clinical and public health burden of skin and soft tissue infections (SSTIs) (3).

The emergence and rapid spread of multidrug-resistant *E. coli* pose a significant global health challenge, limiting the effectiveness of conventional antibiotics and contributing to increased morbidity, mortality, and healthcare costs (4,5). As resistance rates escalate, there is an urgent need to identify alternative, safe, and affordable antimicrobial agents.

Medicinal plants have become a major focus of research due to their rich diversity of bioactive compounds and their long history of use in traditional medicine (6). Plant-derived phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolics, have demonstrated antibacterial activity against MDR bacteria through a variety of mechanisms, such as disrupting bacterial cell walls and modulating resistance pathways (7,8). Furthermore, several studies have shown that medicinal plant extracts can enhance the efficacy of conventional antibiotics when used in combination, indicating their potential role in integrated antimicrobial strategies (8).

Cymbopogon citratus (lemongrass) is an aromatic plant widely used in culinary, cosmetic, and ethnomedicinal applications. Previous studies have demonstrated that its essential oils and extracts contain bioactive constituents such as citral, geraniol, limonene, and myrcene, which exhibit antimicrobial, anti-inflammatory, and antioxidant properties (4). These phytochemicals are known to exert antimicrobial effects through mechanisms such as disruption of microbial cell membranes, inhibition of enzymatic activity, and interference with quorum sensing pathways (4). However, despite these promising attributes, there are limited scientific data specifically evaluating its effectiveness against MDR bacterial strains, which represent one of the most critical threats to current antimicrobial therapies (5). This gap highlights the need for targeted investigations assessing its activity against MDR pathogens.

Laboratory experimental studies have shown that *C. citratus* extracts and essential oil have antibacterial properties due to the presence of citral in the essential oil, making it a strong antibacterial against several bacteria including *S. aureus, Bacillus subtilis, P. aeruginosa, Streptococcus pneumoniae, Streptococcus pyogenes, Neisseria gonorhoeae, Clostridium perfringens, Pseudomonas fluorescens, Acinetobacter baumannii, Aeromonas veronii biogroup sober, Enterobacter faecalis, K. pneumo-*

niae, Salmonella enterica subsp *enterica* serotype *typhimurium, Serratia marcescens, Proteus mirabilis, Shigella flexneri, and Salmonella* Typhi (9).

In-silico analysis refers to the utilization of computer-based tools to investigate biological or chemical interactions, as opposed to traditional laboratory experiments. This approach plays a critical role in modern drug discovery by enabling the identification, screening, and rational design of potential drug candidates through computational techniques (10, 11). Research indicates that drug development is significantly enhanced by *in-silico* investigations because these methodologies allow rapid virtual screening, molecular modeling, and prediction of therapeutic potential prior to experimental validation. In antimicrobial research, computational strategies such as molecular docking provide valuable insights into ligand–target interactions, thereby supporting the evaluation of the antimicrobial potential of *C. citratus* against resistant pathogens (11).

Furthermore, *in-silico* methods facilitate toxicity and pharmacokinetic prediction through absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis, which helps forecast drug-likeness, bioavailability, and possible off-target effects (5,10). By identifying potentially harmful effects early in the development process, these computational tools significantly reduce time, laboratory workload, and financial costs. The integration of molecular docking and ADMET prediction into antimicrobial research therefore enhances drug quality and increases the probability of success fully harnessing the bioactive compounds of *C. citratus* for innovative therapeutic strategies against antimicrobial resistance (AMR).

This study aims to identify the major chemical constituents of *C. citratus* through an exhaustive search of established online databases and to determine the binding affinities and interaction profiles of these compounds against specific *E. coli* protein targets. It seeks to assess the pharmacokinetic properties of the top-performing phytochemicals using computational tools, in order to distinguish potential lead compounds that exhibit both strong binding potency and favorable safety and metabolic profiles. Ultimately, the study evaluates the overall suitability of these bioactive constituents for further development as effective antimicrobial agents.

Materials and method:

Study design:

This study employed an *in-silico* research design to investigate the antimicrobial potential of *C. citratus* against drug-resistant *E. coli.* Molecular docking techniques were applied to predict the binding affinity and interaction patterns between the bioactive compounds of *C. citratus* and selected *E. coli* target proteins. The methodology includes ligand selection and preparation, *E. coli* protein target preparation, molecular docking, interaction analysis, and ADMET screening. Furthermore, ADMET screening was conducted to assess the drug-likeness and safety profile of the identified compounds.

Identification of bioactive compounds of *C. citratus* from databases:

The phytochemical constituents of *C. citratus* were identified through a comprehensive literature review and validated phytochemical databases (PubMed). The chemical structures of selected bioactive compounds were obtained in SDF format from a publicly accessible chemical database (PubChem). These compounds were selected based on their reported antimicrobial activity and structural relevance.

QSAR modeling:

Molecular parameters were derived from the optimized compounds and subsequently used in the development of a valid and reliable quantitative structure-activity relationship (QSAR) model. The calculated molecular para- meters functioned as independent variables, while the experimental inhibitory concentration (IC_{50}) values were designated as the dependent variable. The development of the QSAR model was conducted using the Genetic Function Approximation (GFA) method, as implemented in Materials Studio software (15). The resulting Multiple Linear Regression–GFA (MLR–GFA) model was articulated in the following equation: Predicted $IC_{50} = \alpha + \beta_1X_1 + \beta_2X_2 + ... + \beta_nX_n$ where α denotes the regression constant, X_1, X_2, ... X_n represent the molecular parameters, and β_1, β_2, ... β_n are the respective regression coefficients.

Molecular docking:

Protein targets associated with antimicrobial resistance and bacterial survival in *E. coli* were identified through literature review and sourced from the RCSB Protein Data Bank (PDB). Only proteins with co-crystallized structures resolved by X-ray diffraction at 1.5–2.5 Å were included to ensure high structural fidelity. Proteins from other organisms or lacking crystallographic data were excluded. The three-dimensional structures of ligands were retrieved from PubChem in SDF format, converted to PDB using Open Babel in PyRx workspace, and energy minimization was done to achieve

stable conformations before conversion to PDBQT for docking. Protein preparation involved removing water molecules, co-crystallized ligands, and heteroatoms, followed by the addition of polar hydrogens and conversion to PDBQT format using AutoDock tools.

Docking was performed using AutoDock Vina in the PyRx workspace. Grid box parameters were set for each target protein: 1JL2 (X: 35.95 Å, Y: 46.97 Å, Z: 44.26 Å; center X: 11.46 Å, Y: 3.20 Å, Z: 24.33 Å), 8XZU (X: 59.15 Å, Y: 53.33 Å, Z: 50.31 Å; center X: -8.72 Å, Y: -16.60 Å, Z: -7.49 Å), and 8EY4 (X: 38.54 Å, Y: 33.95 Å, Z: 45.87 Å; center X: 12.26 Å, Y: 9.97 Å, Z: 16.59 Å). Ligand orientations were scored based on forcefield calculations; the top binder was identified. Docked complexes were visualized in BIOVIA Discovery Studio 2025 (Dassault Systèmes, Vélizy-Villacoublay, France), generating 2D and 3D representations of molecular interactions to evaluate binding profiles.

ADMET screening:

The ADMET properties of the selected compounds were assessed using online prediction tools, specifically ADMETlab 3.0. The parameters evaluated included oral bioavailability, permeability across the blood-brain barrier, interactions with cytochrome P450, and toxicity. Drug-likeness was determined based on molecular weight, the number of hydrogen-bond donors and acceptors, and lipophilicity (LogP). ADMETlab 3.0 employs canonical SMILES inputs to deliver highly accurate predictions of physicochemical profiles, pharmacokinetics and toxicity. By utilizing comprehensive databases, the platform evaluates essential metrics, including drug-likeness, bioavailability, and therapeutic potential, for one or more query compounds.

Results

Molecular docking results:

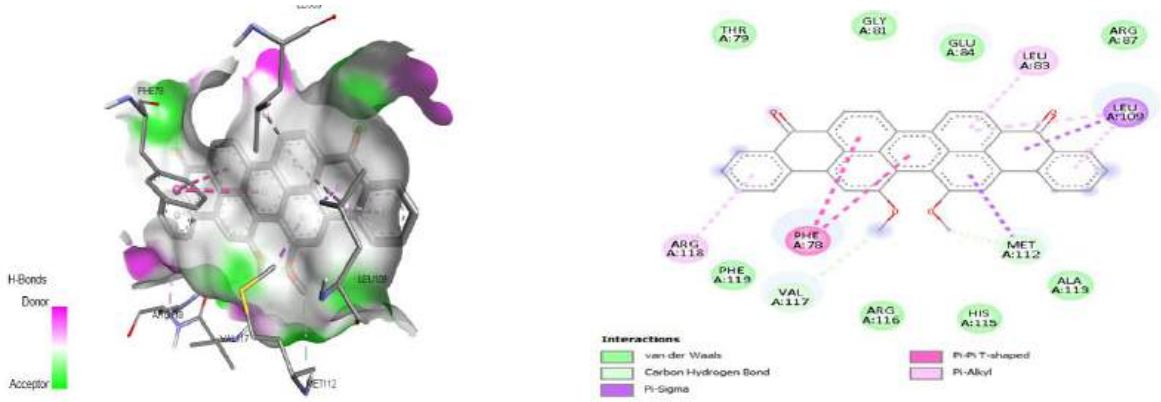
The molecular docking analysis of *C. citratus* offers an in-depth examination of the interactions between its phytochemicals and three specific protein structures of *E. coli*. Each table delineates a unique target, elucidating which compounds exhibit the greatest potential for bacterial inhibition. The analysis of the docking simulations indicates that Ligand 16,17-Dimethoxyviolanthrone is the most effective constituent from the lemon grass extract, demonstrating a binding affinity of -9.2 kcal/mol toward the *E. coli* 1JL2 protein (Table 1, Fig 1).

This high energy score signifies a notably stable protein-ligand complex, significantly surpassing other competitive compounds such as Ligand 11-Nor-9-carboxy-delta9-tetrahydroxycannabinol and Ligand Gossypol which displayed affinities of -7.2 and -6.9 kcal/mol, respectively. Although Ligands Butylated hydroxytoluene and Tymazoline exhibited more moderate interactions, with affinities ranging from -5.8 to -5.5 kcal/mol, the comprehensive data underscore Ligand 16,17-Dimethoxyviolanthrone as the foremost candidate for inhibiting this particular bacterial target (Table 1).

Table 1: Binding Results of *Cymbopogon citratus* phytochemical with 1JL2_*Escherichia coli* protein target

S/N	Ligands	Binding affinity	rmsd/ub	rmsd/lb
1	1JL2_*E. coli*_Cannabinor_uff_E=980.84	-6.2	0	0
2	1JL2_*E. coli*_Butylated hydroxytoluene_uff_E=304.14	-5.8	0	0
3	1JL2_*E. coli*_Dronabinol_uff_E=235.27	-6.4	0	0
4	1JL2_*E. coli*_Dexanabinol_uff_E=339.23	-6.4	0	0
5	1JL2_*E. coli*_11-Nor-9-carboxy-delta9-tetrahydroxycannabinol_uff_E=323.17	-7.2	0	0
6	1JL2_*E. coli*_16,17-Dimethoxyviolanthrone_uff_E=779.90	-9.2	0	0
7	1JL2_*E. coli*_Tymazoline_uff_E=283.52	-5.5	0	0
8	1JL2_*E. coli*_Gossypol_uff_E=866.62	-6.9	0	0
9	1JL2_*E. coli*_2-amino-4-phenyl-4Hpyrano(3,2-H)quinoline-3-carbonitrile_uff_E=241.32	-6.8	0	0
10	1JL2_*E. coli*_2-((1S,6S)-3-Methyl-6-(Prop-1-En-2-Yl)Cyclohex-2-Enyl)-5-Pentylbenzene-1,3-Diol_uff_E=215.14	-6.2	0	0

1JL2: Crystal structure of TCEO RNase H-a chimera combining the folding core from *Thermus thermophilus* RNase H and the remaining region of *Escherichia coli* RNase H (12): msd: root mean square deviation; ub: upper bound; lb: lower bound



(1a) (1b)

Fig 1: (a) 3D and (b) 2D binding interaction of 16, 17- dimethoxyviolanthrone with 1JL2 of *Escherichia coli*

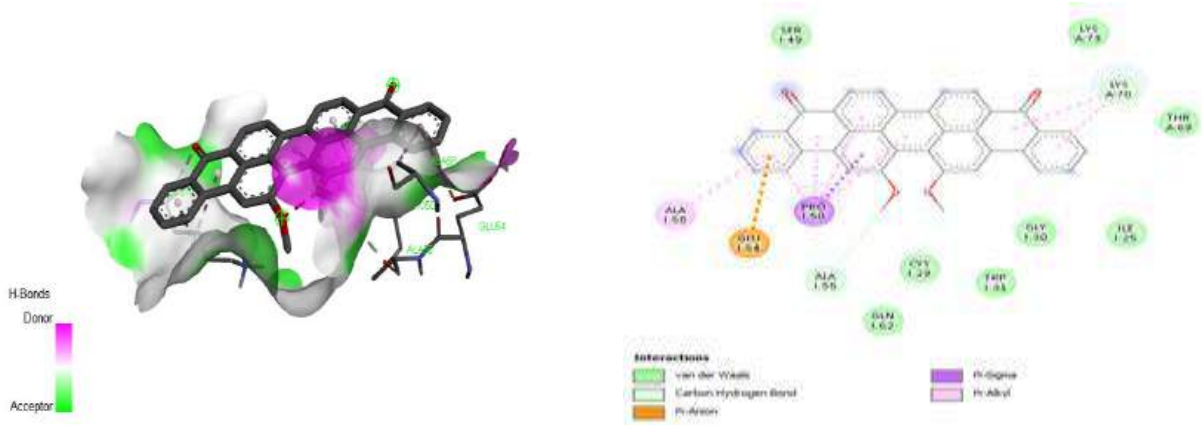
In the examination of the 8EY4 protein target, Ligand 16,17-Dimethoxyviolanthrone consistently exhibited superior inhibitory potential, demonstrating a high binding affinity of -9.1 kcal/mol (Table 2, Fig 2). This sustained performance is further supported by the notable results of Ligand 11-Nor-9-carboxy-delta 9-tetrahydroxycannabinol and Ligand Lenabasum which achieved affinities of -7.9 kcal/mol and -7.7 kcal/mol, respectively.

Conversely, this particular target also recorded the lowest affinity observed in the study, with Ligand 10346 attaining only -5.0 kcal/mol. This finding suggests that the binding pocket of 8EY4 may possess a more restrictive geometry or a chemical environment that is less compatible with certain phytochemical structures in comparison to other proteins analyzed (Table 2).

Table 2: Binding Results of *Cymbopogon citratus* phytochemicals with 8EY4_*Escherichia coli* protein target

S/N	Ligands	Binding affinity	rmsd/ub	rmsd/lb
1	8EY4_*E. coli*_Cannabinor_uff_E=980.84	-6.3	0	0
2	8EY4_*E. coli*_Butylated hydroxytoluene_uff_E=304.14	-5	0	0
3	8EY4_*E. coli*_Dronabinol_uff_E=235.27	-6	0	0
4	8EY4_*E. coli*_Dexanabinol_uff_E=339.23	-7	0	0
5	8EY4_*E. coli*_11-Nor-9-carboxy-delta9-tetrahydroxycannabinol_uff_E=323.17	-7.9	0	0
6	8EY4_*E. coli*_16,17-Dimethoxyviolanthrone_uff_E=779.90	-9.1	0	0
7	8EY4_*E. coli*_Tymazoline_uff_E=283.52	-6	0	0
8	8EY4_*E. coli*_Lenabasum_uff_E=328.21	-7.7	0	0
9	8EY4_*E. coli*_Bromothymol Blue_uff_E=891.42	-6.5	0	0
10	8EY4_*E. coli*_6-Nitrosothymol_uff_E=112.46	-5.5	0	0

8EY4: Contact-dependent growth inhibition toxin-immunity protein complex from Escherichia coli O32:H37 (13); msd: root mean square deviation; ub: upper bound; lb: lower bound



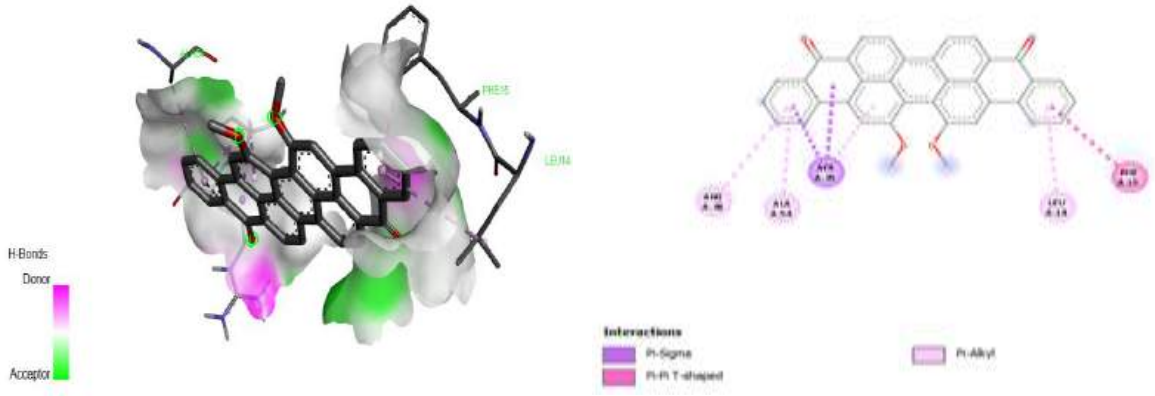
(2a) (2b)

Fig 2: (a) 3D and (b) 2D binding interaction of 16, 17- dimethoxyviolanthrone with 8EY4 of *Escherichia coli*

Table 3: Binding Results of *Cymbopogon citratus* phytochemicals with 8XZU_*Escherichia coli* protein target

S/N	Ligands	Binding affinity	rmsd/ub	rmsd/lb
1	8XZU_*E. coli*_Cannabinor_uff_E=980.84	-7.9	0	0
2	8XZU_*E. coli*_16,17-Dimethoxyviolanthrone_uff_E=779.90	-9.7	0	0
3	8XZU_*E. coli*_Dronabinol_uff_E=235.27	-6.9	0	0
4	8XZU_*E. coli*_Dexanabinol_uff_E=339.23	-7.4	0	0
5	8XZU_*E. coli*_Butylated hydroxytoluene_uff_E=304.14	-6.5	0	0
6	8XZU_*E. coli*_Imlunestrant_uff_E=859.08	-7.9	0	0
7	8XZU_*E. coli*_11-Nor-9-carboxy-delta9-tetrahydrocannabinol_uff_E=323.17	-7	0	0
8	8XZU_*E. coli* o-Thymotic acid_uff_E=158.94	-5.3	0	0
9	8XZU_*E. coli*_Thymol Iodide_uff_E=472.35	-7	0	0
10	8XZU_*E. coli*_10-Isobutyryloxy-8,9-epoxythymol isobutyrate_uff_E=1624.85	-6.2	0	0

8XZU: Crystal structure of N-terminal deletion mutant of Staphylococcal atypical two-cysteine thiol peroxidase complexed with substrate analog (14); msd: root mean square deviation; ub: upper bound; lb: lower bound



(3a) (3b)

Fig 3: (a) 3D and (b) 2D binding Interaction of 16, 17- dimethoxyviolanthrone with 8XZU of *Escherichia coli*

The interactions with the 8XZU protein suggest that it is the most receptive target among the three proteins examined, as demonstrated by Ligand 16,17-Dimethoxyviolanthrone achieving a peak binding affinity of -9.7 kcal/mol (Table 3, Fig 3). This protein exhibits a significant binding capacity with several other ligands, with Ligands Cannabinor and Imlunestrant both achieving a commendable binding score of -7.9 kcal/mol. Additionally, mid-range ligands such as Dexanabinol and Thymol Iodide displayed stronger binding affinities of -7.4 kcal/mol and -7.0 kcal/mol, respectively, compared to their counterparts in prior assessments. This finding positions 8XZU as a primary site for potential antimicrobial action, attributable to its highly compatible binding pocket (Table 3).

The Root Mean Square Deviation (RMSD) values across all three tables consistently measured at 0, thereby confirming that the data accurately reflects the optimal docking pose for each compound. A comparative analysis of the three targets reveals that while the ligands derived from *C. citratus* demonstrate general efficacy, their potency is particularly enhanced when directed towards the 8XZU protein, with compound 16,17-Dimethoxyviolanthrone consistently identified as the leading candidate for further investigation.

Physicochemical and ADMET properties of the lead compound:

The physicochemical and ADMET properties of the lead compound, 16,17-dimethoxyviolanthrone, is presented in Table 4. The compound presents considerable challenges for oral drug delivery, attributed to its high lipophilicity (logP, with an optimal range of 0-3) and its extremely low solubility (logS of -11.965), which reflects poor aqueous solubility. Furthermore, it exhibits a low Quantitative Estimate of Drug-likeness (QED of 0.172), suggesting that it is deemed overly complex. It demonstrates optimal Caco-2 permeability, suggesting efficient translocation through tissue cells; however, it exhibits low bioavailability, indicating inadequate oral absorption attributable to first-pass metabolism. Furthermore, the high level of P-glycoprotein (P-gp) inhibition raises concerns regarding potential drug-drug interactions, as it may influence the efficacy of concomitantly administered medications.

Table 4: Physicochemical and ADMET properties of the lead compound (16,17- dimethoxyviolanthrone)

Compound	Properties					
16,17- dimethoxyviolanthrone	**Physicochemical properties**					
	Molecular weight	logP	logS	TPSA	nHA/nHD	QED
	516.14	7.573	-11.965	52.6	4.0/0.0	0.172
	Absorption					
	Caco-2 permeability	HIA	Pgp-inhibitor	Bioavailability		
	-4.726	0.045	0.992	0.935		
	Distribution					
	PPB	VDss	BBB penetration			
	98.805%	0.603 L/kg	0.252			
	Metabolism					
	CYP1A2 inhibitor	CYP3A4 substrate	CYP2C9 inhibitor			
	0.957	0.697	0.817			
	Excretion					
	Plasma clearance	Half-life ($T_{1/2}$)				
	4.16	0.512				
	Toxicity					
	hERG blockers	Hepatotoxicity	Mutagenicity	Rat oral toxicity	Carcinogenicity	
	0.983	0.988	0.106	0.237	0.826	

The assessment of Human Intestinal Absorption (HIA) indicates a low likelihood of suboptimal intestinal absorption, predicting a human intestinal absorption rate of 30% or greater for the compound. The compound has a notably high protein binding affinity, exceeding the optimal threshold of 90%. This observation suggests a potential low therapeutic index, as a reduced amount of free drug is accessible to reach the intended target due to the substantial proportion of the drug binding to plasma proteins. The volume of distribution at steady state (VDss) suggests a uniform distribution throughout the tissues. Furthermore, the compound's limited ability to penetrate the blood-brain barrier (BBB) implies a reduced distribution to the central nervous system, which may correlate with a diminished risk of central nervous system-related adverse effects.

16,17-dimethoxyviolanthrone demonstrated significant predicted inhibition of CYP 1A2 and CYP2C9, indicating a substantial risk of drug-drug interactions and a potential for reduced metabolism of other drugs that are processed by these enzymes. Furthermore, it is probable that the compound will be metabolized by CYP3A4, which is recognized as the predominant enzyme involved in drug metabolism within the liver. It showed a moderate plasma clearance rate and possesses a short half-life, suggesting that more frequent dosing may be necessary to sustain therapeutic levels. Finally, it presents notable safety concerns, specifically regarding hepatotoxicity, cardiotoxicity, and carcinogenicity. This indicates that the compound is detrimental in its existing formulation.

Discussion:

Antimicrobial resistance is a significant issue in modern medicine, especially with the increase of multidrug-resistant *E. coli* infections, which has made many standard antibiotics less effective. The global health impacts of infections caused by MDR *E. coli* have been highlighted by various clinical studies that show widespread resistance and emphasize the urgent need to investigate alternative antibacterial agents (16).

In this study, *in-silico* methods, including molecular docking and ADMET prediction, were employed to assess selected phytochemicals from *C. citratus* for their potential to inhibit important *E. coli* resistance-related proteins. The docking results showed that 16,17-dimethoxyviolanthrone exhibited the strongest binding affinities for several target proteins, including the 8XZU protein, which might indicate a high degree of compatibility within the protein's active site. These findings are in agreement with broader research that suggests computational screening can effectively identify phytomolecules that have strong protein-ligand binding potential against drug-resistant bacterial targets (10).

The strong binding affinities observed in this study are seen as indicators of possible antimicrobial action *in-silico* (11). However, it is important to note that while molecular docking can predict favorable interactions, these results must be validated through experimental methods since computational binding scores do not fully encompass biological complexities. Additional experimental research supports the antimicrobial potential of *C. citratus* extracts. For example, a 2025 *in vitro* study indicated significant zones of inhibition against *E. coli* and other pathogens when lemongrass leaf extracts were used, thereby confirming the antibacterial properties suggested by the computational models (17). Another recent study found that essential oils from *C. citratus* showed disruptive activity against *E. coli* biofilms, which is an important mechanism for overcoming bacterial defenses and persistence (18). These experimental findings reinforce the idea that phytochemicals from lemongrass have substantial biological activity, justifying their exploration as antimicrobial agents.

Despite the promising anti-*E. coli* activity of *C. citratus* phytochemicals in this study, the ADMET predictions showed concerns related to pharmacokinetics and toxicity. The lead compound demonstrated high lipophilicity and poor solubility, indicated by high log P and low log S values, which might pose challenges for bioavailability. Additionally, the predicted risks of hepatotoxicity and cardiotoxicity from hERG (human ether-à-gogo-related gene) inhibition point toward potential safety issues. These predicted liabilities are consistent with findings from toxicological evaluations where lemongrass extracts exhibited mild hepatotoxic effects at higher doses, underscoring the necessity for careful dose exploration and safety profiling (17).

A promising direction noted in recent literature is the combination of phytochemicals with conventional antibiotics to achieve synergistic effects and diminish resistance development (19). This approach reflects a growing interest in combination therapies that can enhance antimicrobial effectiveness while potentially reducing the necessary doses of toxic compounds.

Conclusion:

This *in-silico* study identified 16,17-dimethoxyviolanthrone of *C. citratus* with strong docking performance, suggesting potential as a lead compound against drug-resistant *E. coli.* However, ADMET analysis highlighted significant pharmacokinetic and safety concerns, including hepatotoxicity, cardiotoxicity, carcinogenicity, and possible drug–drug interactions. Overall, while *C. citratus* demonstrates promise as a source of novel antimicrobial agents, further structural modification and optimization are necessary to improve safety, metabolic stability, and therapeutic suitability before clinical development for antimicrobial-resistant infections.

To enhance the therapeutic potential of bioactive compounds, future research should prioritize structural modification and optimization. By concentrating on derivatives of lead compounds such as 16,17-dimethoxyviolanthrone, medicinal chemists can address identified risks, including hepatotoxicity and cardiotoxicity. This endeavor requires a careful balance of modifying the chemical scaffold to diminish adverse effects while simultaneously preserving or ideally enhancing the compound's antimicrobial efficacy against target pathogens.

Moreover, it is imperative to move beyond computational modeling to ensure clinical relevance. Although current *in silico* findings are encouraging, rigorous *in vitro* and *in vivo* experimental validation is essential as the subsequent step. Laboratory experiments and animal models will be crucial for confirming the actual antimicrobial effectiveness and physiological safety of these *C. citratus* phytochemicals when confronted with the complexities of living biological systems, particularly with respect to drug-resistant *E. coli*.

Additionally, addressing the safety profile and metabolic challenges identified during ADMET screening is of utmost importance. Specialized studies should examine the risks of drug-drug interactions associated with compounds that exhibit strong inhibition of enzymes such as CYP1A2 and CYP2C9. To mitigate these risks and compensate for short half-lives or specific clearance rates, subsequent research should investigate dosage and formulation strategies. The development of advanced drug delivery systems could be instrumental in maintaining therapeutic concentrations within the body and ensuring a stable metabolic profile.

Finally, the scope of research should be expanded to investigate the synergistic and broad-spectrum potential of these extracts. Testing these phytochemicals against a broader range of drug-resistant pathogens beyond *E. coli* will elucidate the full utility of lemongrass-derived compounds. Furthermore, exploring the synergy between different phytochemicals or their combinations with existing antibiotics could unveil innovative strategies for combating AMR and rejuvenating the current pharmaceutical arsenal.

Contributions of authors:

PD supervised the study; FO performed the *in-silico* analysis; and KP co-supervised the study.

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Authors declare no conflicts of interest.

Ethics statement and informed consent:

This study did not involve human participants, animal samples, or materials requiring ethical approval. As no human participant were involved, informed consent was not applicable.

Artificial Intelligence (AI) use disclosure:

Generative AI, Quillbot and Grammarly, were used in this study for rephrasing and spell checks. My bib was used to arrange the reference. Google Gemini was used to assist in the interpretation and clarification of results. The authors reviewed, edited, and validated all outputs to ensure accuracy and scientific integrity.

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Original Article **Open Access**

Exploring the antimicrobial potential of *Azadirachta indica* (Neem) phytoconstituents against drug-resistant *Acinetobacter baumannii*: A molecular docking and ADMET screening study

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Abstract:

Background: The emergence of multidrug resistant (MDR) Acinetobacter baumannii remains at the top of the health care concern around the world which thereby emphasizes the need to discover novel antimicrobials from medicinal plants, such as *Azadirachta indica* (neem) in treatment of infections caused by MDR A. baumannii. The current in-silico study was aimed to evaluate inhibitory potential of Neem derived phytoconstituents against essential proteins of *A. baumannii* and their evaluation of pharmacokinetic characteristics.
Methodology: Selected bioactive constituents of *A. indica* extracted from proteomic databases were subjected to systematic molecular docking protocol (using Auto Dock Vina in PyRx interface) against three important *A. baumannii* protein targets; 4Y0A, 5YDB and 4DXV crystallographic structures extracted from PubChem database. The resulting binding affinity values along with detailed insights into the landscape of ligand-protein interactions provided information on the inhibitory potential of each compound. Thereafter, the highest-ranking candidates by their docking values were subjected to ADMET profiling (using ADMETlab 3.0), which entailed evaluation of absorption, distribution, metabolism, excretion, and toxicity (ADMET), *in silico* to assess their pharmaceutical suitability and prospective clinical application.
Results: Through rigorous molecular docking studies, several phytochemicals were extracted from *A. indica* proteomic databases, which exhibited significant high binding affinities against the three key proteins targets of *A. baumannii*. Among these, isonimocinolide, nicotiflorin, and nimbaflavone showed the highest activity with binding energy ranging from -8.7 to -9.9 (kcal/mol), thus suggesting significant potential for inhibition. However, assessments of ADMET profile indicated major limitations of the pharmacokinetic and toxicological nature. Although the metabolic interactions obtained were acceptable for some of the compounds, *in-silico* predictions suggest considerably low oral bioavailability for the three phytochemical compounds. Furthermore, toxicological screening outlined possible hepatotoxic as well as genotoxic effects.
Conclusion: In this *in-silico* study, computational analysis showed that some *A. indica* phyto- constituents have significant inhibitory activity against MDR *A. baumannii* protein targets. However, low oral bioavailability, and large tolerance risk such as liver damage and genotoxicity, indicate the need for improvement in the structures of the phytochemicals. Although these neem derived phytochemicals show promising inhibitory activity, systematic optimization with rigorous *in vitro* validation will be necessary to improve their safety profiles and therapeutic efficacy.

Keywords: *Azadirachta indica; Acinetobacter baumannii*; molecular docking; ADMET; AMR; phytochemicals

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Exploration du potentiel antimicrobien des phytoconstituants d'*Azadirachta indica* (Neem) contre *Acinetobacter baumannii* résistant aux antibiotiques: étude de docking moléculaire et de criblage ADMET

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Résumé:

Contexte: L'émergence d'*Acinetobacter baumannii* multirésistant (MDR) demeure une préoccupation majeure de santé publique à l'échelle mondiale, soulignant ainsi la nécessité de découvrir de nouveaux antimicrobiens issus de plantes médicinales, telles qu'*Azadirachta indica* (Neem), pour le traitement des infections causées par *A. baumannii* MDR. La présente étude *in silico* visait à évaluer le potentiel inhibiteur de phytoconstituants dérivés du neem sur des protéines essentielles d'*A. baumannii*, ainsi que leurs caractéristiques pharmacocinétiques.
Méthodologie: Des constituants bioactifs sélectionnés d'*A. indica*, extraits de bases de données protéomiques, ont été soumis à un protocole de docking moléculaire systématique (à l'aide d'AutoDock Vina dans l'interface PyRx) contre trois cibles protéiques importantes d'*A. baumannii*: les structures cristallographiques 4Y0A, 5YDB et 4DXV, extraites de la base de données PubChem. Les valeurs d'affinité de liaison obtenues, associées à une analyse détaillée des interactions ligand-protéine, ont permis d'évaluer le potentiel inhibiteur de chaque composé. Les candidats les plus prometteurs, selon leurs valeurs d'amarrage moléculaire, ont ensuite fait l'objet d'un profilage ADMET (à l'aide d'ADMETlab 3.0), comprenant l'évaluation *in silico* de l'absorption, de la distribution, du métabolisme, de l'excrétion et de la toxicité (ADMET), afin d'évaluer leur potentiel pharmaceutique et leur application clinique potentielle.
Résultats: Grâce à des études rigoureuses d'amarrage moléculaire, plusieurs composés phytochimiques extraits des bases de données protéomiques d'*A. indica* ont présenté des affinités de liaison élevées et significatives pour les trois protéines cibles clés d'*A. baumannii*. Parmi ceux-ci, l'isonimocinolide, la nicotiflorine et la nimbaflavone ont montré l'activité la plus importante, avec une énergie de liaison comprise entre -8,7 et -9,9 kcal/mol, suggérant ainsi un potentiel d'inhibition significatif. Cependant, l'évaluation du profil ADMET a révélé des limitations majeures d'ordre pharmacocinétique et toxicologique. Bien que les interactions métaboliques obtenues soient acceptables pour certains composés, les prédictions *in silico* suggèrent une biodisponibilité orale considérablement faible pour les trois composés phytochimiques. De plus, le criblage toxicologique a mis en évidence des effets hépatotoxiques et génotoxiques potentiels.
Conclusion: Dans cette étude *in silico*, l'analyse computationnelle a montré que certains constituants phytochimiques d'*A. indica* présentent une activité inhibitrice significative contre les cibles protéiques d'*A. baumannii* multirésistant. Cependant, la faible biodisponibilité orale et le risque élevé d'effets indésirables, tels que des lésions hépatiques et une génotoxicité, indiquent la nécessité d'améliorer la structure de ces composés phytochimiques. Bien que ces composés phytochimiques dérivés du neem présentent une activité inhibitrice prometteuse, une optimisation systématique, associée à une validation in vitro rigoureuse, sera nécessaire pour améliorer leur profil de sécurité et leur efficacité thérapeutique.

Mots-clés: *Azadirachta indica*; *Acinetobacter baumannii*; amarrage moléculaire; ADMET; AMR; phytochimiques

Introduction:

Antimicrobial resistance (AMR) has become a pre-eminent global health challenge of the twenty-first century. These are changes in microorganisms due to mutations resulting in resistance to conventional therapeutic agents. The increase in the rates of infection due AMR, together with a lack of new development of new antimicrobial drugs, has led to a critical public health gap (1). The projections suggest that the current toll of 700 000 deaths a year may rise to 10 million by 2050, and this will be accompanied by a staggering rise in healthcare expenditure (2).

While antibiotics are indispensable in clinical practice (instrument), inappropriate and prolonged use have imposed such strong selective pressure leading to the emergence of resistant bacterial phenotypes (3). In response, the World Health Organization (WHO) revised the Bacterial Priority Pathogens List (BPPL) in 2024 and outlines 24 organisms that are high, critical and medium priority in response to the need to guide research and development efforts towards the most important threats (4).

Among the most formidable organisms in the 'critical' category is *Acinetobacter baumannii*, a predominant aetiological agent of nosocomial infections such as ventilator-associated pneumonia and bacteremia. This is enhanced by its ability to form clonal outbreaks, and its frighteningly broad resistance to carbapenem antibiotics (5). Carbapenem-resistance among *A. baumannii* (CRAB) has become a worldwide healthcare burden, associated with prolonged hospital stay, high mortality and significant economic costs (6).

As the need for new antimicrobials re-emerges, interest in medicinal botanicals with known pharmacological content and demonstrated efficacy in the past has been revived. Among these *Azadirachta indica* commonly known as Neem, belonging to Meliaceae family, emerges as well documented source of diverse bioactive constituents (7). Its efficacy against both Gram-positive and Gram-negative, bacterial strains is attributed to its species of phytochemicals such as nimbin, nimbidin, azadiraitin and gedunin with pronounced bacteriostatic and bactericidal effects. Owing to its rich source of phenolic, flavonoid and terpenoid, Neem is a

promising natural source of hits for the rational design of new drug scaffolds to fight multidrug resistant infections (8).

While the antimicrobial potential of *A. indica* is well documented, there are still large knowledge gaps in understanding the interaction between individual neem-derived phytochemicals and drug-resistant mediators in bacteria, most notably in *A. baumannii.* The current study was aimed at determining the potential inhibitory activity of the phytochemical constituents of neem on a set of clinically relevant protein targets of *A. baumannii* (4Y0A, 5YDB and 4DXV), using structure-based molecular docking. The pharmacokinetic compatibility and toxicity risk of the phytochemicals were further evaluated using complimentary assessment of ADMET profiling.

Materials and method:

Study design:

This research is an *in-silico* study to evaluate the antimicrobial efficacy of *A. indica* against MDR *A. baumannii*. The research followed a computational workflow i. e. bioactive Neem ligands and essential bacterial target proteins first extracted from proteomic databases and preprocessed for simulation. Later, molecular docking was performed in order to quantify binding affinities (kcal/mol) and describe structural interactions between selected phytochemicals and the bacterial targets. High affinity candidates were then prioritized for comprehensive ADMET screening to assess the viability of the candidates during clinically relevant pharmacokinetic screening tests as well as safety profiles of the candidate compounds. This combined approach was aimed to identify highly potent lead compounds with the best drug likeness for further experimental settings.

Selection of phytochemical compounds and protein targets:

Structures of bioactive compounds of *A. indica* were obtained from PubChem database (9). Three key targets for protein production in *A. baumannii* were obtained from the RSCB Protein Data Bank (PDB), namely 4Y0A, 5YDB and 4DXV (10).

Data collection and measurements:

Molecular docking simulation was carried out using Auto Dock vina software in PyRx environment version (v 0.8), which was subsequently visualized and scrutinized for the interactions of proteins and ligand compounds using BIOVIA Discovery Studio version 2025. The pharmacokinetic and toxicological characteristics of the best ligands were predicted in ADMETlab web 3.0 server (11). The results were expressed in terms of ranked inhibitory efficacy using binding free energies that were reported in kcal/mol, while toxicity metrics were represented in terms of probability indices ranging from 0.0 to 1.0.

QSAR modeling:

A Quantitative Structure-Activity Relationship (QSAR) structure was built for correlating the molecular descriptors of the *A. indica* constituents (independent variables) with the experimental IC50 values (dependent variable). Using Genetic Function Approximation (GFA) tool within Materials Studio, we successfully generated the GFA (Multiple Linear Regression - GFA (MLR - GFA)) equation; Predicted IC_{50} = a + b 1x1 + b 2x2 + b n x n. This model provides a strong validation-based statistical framework tool for predicting neem derivatives inhibitory capacity against *A. baumannii* based on their distinctive molecular characters.

Molecular docking:

The three high resolution protein targets for *A. baumannii* (PDB IDs: 4Y0A, 5YDB, 4DXV) were selected from the RCSB Protein Data Bank based on stringent selection criteria which included the resolution of the X-ray diffraction data (1.5-2.5 A), the co-crystallization status, and the mutational stability. Protein preparation involved removal of heteroatoms and crystalline waters followed by the addition of polar hydrogens using the Auto Dock Tools. The ligand structures extracted from PubChem were energy minimized and then converted to PDBQT format with Open bolt.

Docking calculations were done using Auto Dock Vina software in the PyRx program, using site specific coordinates of grid boxes adapted to active site of each target (for example, center X = -1.69 Y = 8.19 Z = 14.17 for 4Y0A). The resulting binding poses were ranked according to the force field scoring functions, defining Nimbaflavone, Isonimocinolide and Nicotiflorin as the best ligands to the target 4Y0A, 5YDB and 4DXV respectively. Interaction geometries were defined and visualized by using BIOVIA Discovery Studio 2025.

ADMET screening:

The lead phytochemicals were subjected to a stringent ADMET screening using ADMETlab 3.0 computational platform (11). The screening protocol included Lipinski's Rule of Five, oral bioavailability, gastrointestinal absorption, and critical cytochrome P450 enzymes interactions predictions. Safety assessments in-

cluded predictions of the hepatotoxicity, mutagenicity and systemic clearance rates. Molecules that showed both high affinity of binding and positive numbers for safety and drug memorial properties were selected for further experimental verification.

Results:

Molecular docking results:

The detailed results of docking that include binding energies in kcal/mol and the different forms of ligand-receptor interactions between selected phytochemicals from *A. indica* with the three protein structures of *A. baumannii* (4Y0A, 5YDB and 4DXV) are summarized in Tables 1 to 3 corresponding to the individual targets for bacterial protein.

Docking simulation of *A. indica* phytochemicals against 4Y0A protein of *A. baumannii* yielded binding affinities in the range of -4.8 to -8.7 (kcal/mol). Nimbaflavone had the highest binding inhibitor with binding energy of -8.7 (kcal/mol) while meliantriol and beta-sitosterol-beta-D- glucoside had strong binding energy of -7.7 (kcal/mol) and -7.5 (kcal/mol), respectively.

Moderate binding affinities were observed for deacetylnimbin, 28-deoxonibolide and deacetylsalannin within the range -6.5 to -7.0 (kcal/mol), which suggests a fair degree of biological activity. In contrast, squalene and hexamethyltetracosa-hexaene derivatives had lower binding energies (i.e. 4.8 to 5.6 kcal/mol), thereby showed reduced inhibitory ability towards this specific target. All of the ligands tested showed a Root Mean Square Deviation (RMSD) of 0.0A, thus confirming the stability of the ligand orientation and the credibility of the docking poses in the active site of 4Y0A (Table 1).

The molecular docking analysis studies performed on the phytochemicals from *A. indica* against *A. baumannii* 5YDB protein target showed the binding affinity with values from -5.0 (kcal mol) to -9.1 (kcal mol). Among the phytochemicals, isonimocinolide was the most promising one with binding energy of -9.1 (kcal mol), therefore indicating the highest interaction and specificity for bound activation site of the target protein. Additional ligands with great inhibitory potential were Meliantriol (-8.5 kcal/mol), Beta-sitosterol-Beta-D-glucoside (-8.2 kcal/mol) and 28-deoxonimbolide (-8.0 kcal/mol) (Table 2).

Table 1: Binding results of *Azadirachta indica* with 4Y0A_*Acinetobacter baumannii*

S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	4Y0A_Meliantriol_uff_E=997.15	-7.7	0	0
2	4Y0A_Deacetylnimbin_uff_E=982.24	-7.0	0	0
3	4Y0A_2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=248.91	-5.6	0	0
4	4Y0A_(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.94	-5.0	0	0
5	4Y0A_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-7.5	0	0
6	4Y0A_Elemene; delta-Elemene_uff_E=218.48	-5.6	0	0
7	4Y0A_gamma-Elemene_uff_E=179.27	-6.1	0	0
8	4Y0A_(1S,4aS,8aR)-1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene_uff_E=173.65	-6.1	0	0
9	4Y0A_deuterio-squalene_uff_E=190.36	-4.8	0	0
10	4Y0A_(14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-5.2	0	0
11	4Y0A_(18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-5.3	0	0
12	4Y0A_Deacetylsalannin_uff_E=1158.52	-6.5	0	0
13	4Y0A_28-Deoxonimbolide_uff_E=1088.15	-6.7	0	0
14	4Y0A_Nimbaflavone_uff_E=311.89	-8.7	0	0

UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

A moderate binding affinities were observed for the molecules; deacetylsalannin, deacetylnimbin, gamma-murolene, and elemene, which ranged from -6.2 thousand calories (kcal) per mole to -7.9 (kcal/mol). On the other hand, the derivatives of deuterio-squalene and hexamethyl-tetracosahexaene had relatively low inhibitory potential, with affinities ranging from -5.0 (kcal/mol) to -5.7 (kcal/mol). The technical robustness of the docking results was also supported by an RMSD value of 0.0 A for all ligands and provided stable and predictable binding orientation. Accordingly, isonimocinolide piloted series of potent binders were sucked out and prioritized for further ADMET profiling and validation through additional experiments.

Molecular docking of *A. indica* phytochemicals on *A. baumannii* 4DXV protein target showed binding affinity between -4.8 and -9.9 (kcal/mol). Nicotiflorin had the most potent inhibitory activity with a binding energy of -9.9 (kcal/mol). Strong interactions were also shown by Nimbaflavone (with -8.5 kcal/mol), beta-sitosterol-beta-beta-glucoside (-7.7 kcal/mol) and 28-deoxonimbolide (-7.6 kcal/mol).

Moderate affinities were recorded for deacetylsalannin (-7.3kcal/mol) and deacetylnimbin (-6.8kcal/mol). On the contrary, hexamethyltetracosahexaene derivatives and squalene-related compounds exhibited weak to moderate interactions with a value range between (-4.8 to -6.2 kcal mol). Technical validation of these results was confirmed by a Root Mean Square Deviation (RMSD) of 0.0 A within all the ligands which indicates the consistency of the docking orientations and completeness of a reliable binding pose. Overall, nicotiflorin and nimbaflavone were found to be the most promising inhibitors of the 4DXV protein (Table 3).

Table 2: Binding results of *Azadirachta indica* with 5YDB_*Acinetobacter baumannii*

S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	5YDB_Meliantriol_uff_E=997.15	-8.5	0	0
2	5YDB_Deacetylnimbin_uff_E=982.24	-7.4	0	0
3	5YDB_Super Squalene_uff_E=248.91	-6.3	0	0
4	5YDB__(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.94	-5	0	0
5	5YDB_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-8.2	0	0
6	5YDB_ Elemene_uff_E=218.48	-6.2	0	0
7	5YDB_ Elemene_uff_E=179.27	-6.6	0	0
8	5YDB_gamma-Muurolene_uff_E=173.65	-7.2	0	0
9	5YDB_deuterio-squalene_uff_E=190.36	-5.7	0	0
10	5YDB_(14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-6.1	0	0
11	5YDB_(18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-5.4	0	0
12	5YDB_Deacetylsalannin_uff_E=1158.52	-7.9	0	0
13	5YDB_28-Deoxonimbolide_uff_E=1088.15	-8	0	0
14	5YDB_Isonimocinolide_uff_E=1018.00	-9.1	0	0

UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

Table 3: Binding results of *Azadirachta indica* with 4DXV_*Acinetobacter baumannii*

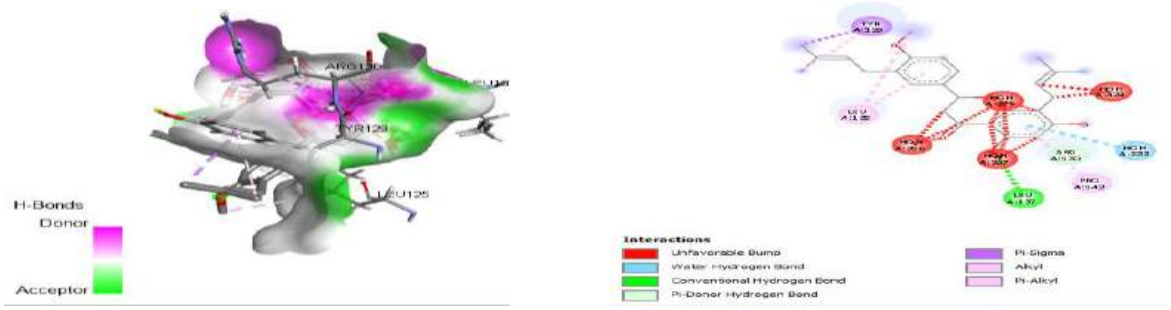
S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	4DXV_Nicotiflorin_uff_E=736.07	-9.9	0	0
2	4DXV_Deacetylnimbin_uff_E=982.24	-6.8	0	0
3	4DXV_2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=248.91	-5.8	0	0
4	4DXV_(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.94	-6.1	0	0
5	4DXV_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-7.7	0	0
6	4DXV_Elemene_uff_E=218.48	-5.7	0	0
7	4DXV_(-)-gamma Elemene_uff_E=179.27	-6.2	0	0
8	4DXV_gamma-Muurolene_uff_E=173.65	-6	0	0
9	4DXV_deuterio-squalene_uff_E=190.36	-5.6	0	0
10	4DXV_(14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-5	0	0
11	4DXV_(18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene_uff_E=254.57	-4.8	0	0
12	4DXV_Deacetylsalannin_uff_E=1158.52	-7.3	0	0
13	4DXV_28 Deoxonimbolide_uff_E=1088.15	-7.6	0	0
14	4DXV_Nimbaflavone_uff_E=311.89	-8.5	0	0

UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

The technical validity of the predicted ligand-protein interactions for all three targets (5YDB, 4DXV and 4Y0A) was confirmed by the Root Mean Square Deviation (RMSD), which showed continuously 0.0 A at the upper and lower limits. Such results represent very stable docking conformations and reliable binding conformations for all ligands tested.

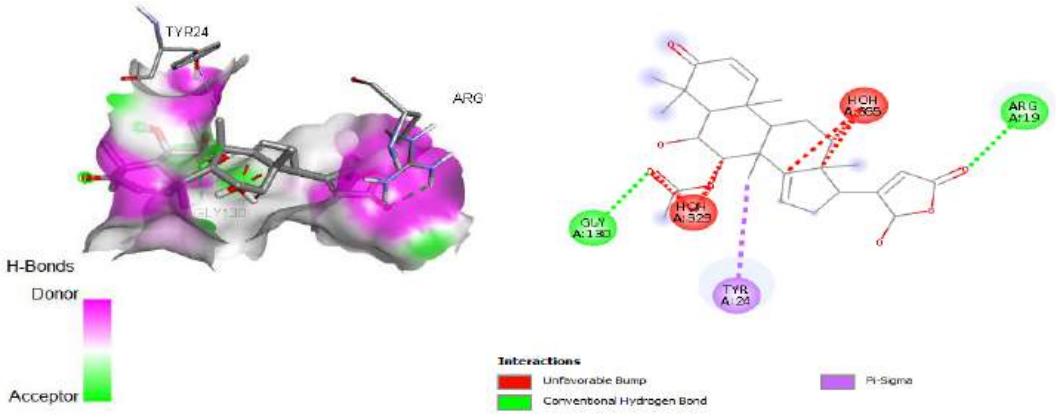
The comparative analysis identified the strongest binders for each protein target; isonimocinolide had the highest affinity ligand for 5YDB protein with an estimated binding free energy of -9.1 (kcal/mol), which left nicotiflorin the strongest ligand for the 4DXV target with a binding free energy of -9.9 (kcal/mol), while nimbaflavone was the most effective ligand for the 4Y0A target with a binding free energy of -8.7 (kcal/mol). All three phytochemicals were consistently ranked at the top of the binding hierarchy that supports their high inhibitory potential.

In summary, the results of docking support that nimbaflavone (Fig 1), isonimocinolide (Fig 2) and nicotiflorin (Fig 3) were the most promising bioactive derivatives of *A. indica*. In response to their binding affinities and stably persistent conformations seen for all of the *A. baumannii* proteins assayed, these compounds were prioritized for further pharmacokinetic and toxicity profiling.



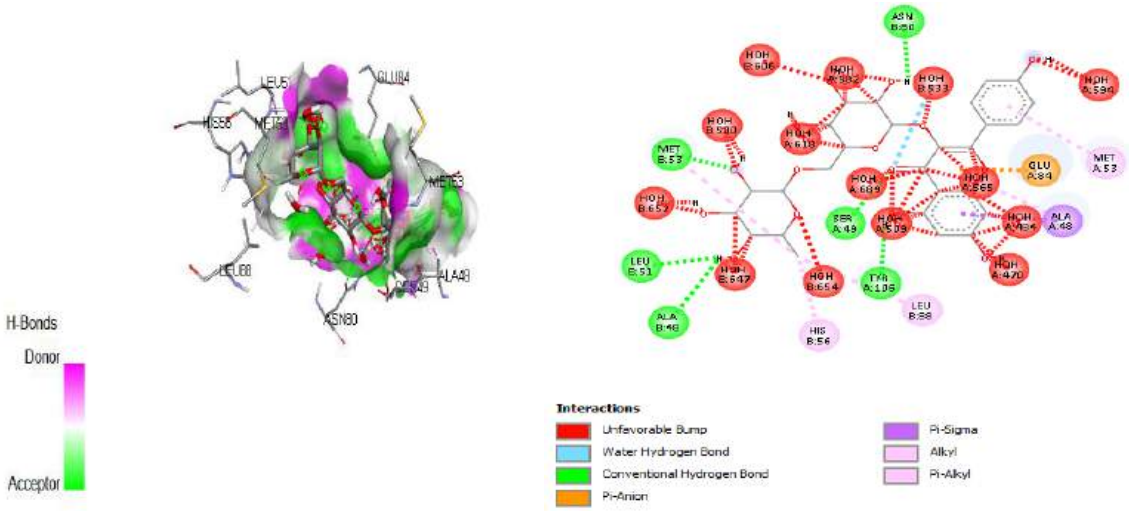
(a) (b)

Fig 1: (a) 3D and (b) 2D binding interaction of nimbaflavone with 4Y0A of *Acinetobacter baumannii*



(a) (b)

Fig 2: (a) 3D and (b) 2D binding interaction of isonimocinolide with 5YDB of *Acinetobacter baumannii*



a b

Fig 3: (a) 3D and (b) 2D binding interaction of nicotiflorin with 4DXV of *Acinetobacter baumannii*

Physicochemical properties of the lead phytochemical compounds of *A. indica*:

The pharmacokinetic and toxicity profiles of the top-scoring ligands were carried out to test their drug-like and oral bioavailability seen in Table 4. Isonimocinolide had the best ADMET profile with acceptable molecular weight, lipophilicity and aqueous solubility. These are favorable characteristics for obtaining a promising balance between intestinal absorption and metabolic stability. On the other hand, nimbaflavone, although meeting many drug likeness criteria, is associated with high lipophilicity and low solubility and this may be associated with poor systemic bioavailability.

Nicotiflorin was found to violate some of Lipinski's Rule of Five criteria, specifically in terms of being high in molecular weight and polar. Such violations would suggest the possibility of membrane permeability problems, as well as sub-optimal oral absorption. Consequently, from the combination of integrated ADMET characteristics, isonimocinolide proves to be the most promising lead compound for further structural optimization and antimicrobial development.

Table 4: Physicochemical properties of the lead phytochemical compounds of *Azadirachta indica*

Compound	M/W	HBA	HBD	TPSA	logP	logS	Structural stability
Nimbaflavone	422.21	5.0	2.0	75.99	6.069	-5.65	Stable
Isonimocinolide	484.25	7.0	2.0	110.13	2.294	-4.514	Moderate/poor
Nicotiflorin	594.16	15.0	9.0	249.2	1.16	-2.557	Stable

ADMET properties of lead phytochemical compounds of *A. indica*:

The ADMET evaluation of the *A. indica* derivatives showed a general low extent of oral bioavailability in the selection of compounds as shown in Table 5. Although the ligands generally showed moderate membrane permeability and intestinal absorption, their predicted interaction as a substrate or inhibitors of P-glycoprotein (P-gp) suggest a significant susceptibility to active efflux which likely will result in reduced systemic absorption.

Of the candidates, nimbaflavone had the most promising pharmacokinetic profile with respect to absorption. Conversely, isonimocinolide and nicotiflorin showed pharmacokinetic determinants related to poor bioavailability to be of concern for their ability to reach therapeutic concentrations using oral administration. These observations reveal the need for the structural improvement or devising of special drug delivery technologies, to generate a clinical viability of these neem-derived antimicrobials.

Table 5: ADMET properties of the lead phytochemical compounds of *Azadirachta indica*

Compound	ADMET properties						
	Absorption						
	CaCO-2 permeability	MDCK permeability	PAMPA permeability	Human intestinal absorption (HIA)	Pgp-inhibitor	Pgp-substrate	Bioavailiability
Nimbaflavone	-4.813	-4.63	0.049	0.22	0.986	0.014	Poor
Isonimocinolide	-5.033	-4.637	0.96	0.059	0.385	0.02	Poor
Nicotiflorin	-6.532	-5.029	1.0	0.137	0.0	0.924	Poor
	Distribution						
	Plasma binding protein (PPB)		Fraction unbound	Volume of distribution	Blood brain barrier penetration		Distribution stability
Nimbaflavone	96.41%		3.375	0.541L/Kg	0.0		Moderate
Isonimocinolide	84.228%		15.366	-0.117 L/Kg	0.168		Moderate/poor
Nicotiflorin	85.118%		13.389	-0.038 L/Kg	0.0		Poor
	Metabolism						
	CYP1A2 inhibitor		CYP3A4 substrate		CYP2C9 inhibitor		
Nimbaflavone	0.008		0.743		1.0		
Isonimocinolide	0.0		0.24		0.0		
Nicotiflorin	0.0		0.O		0.0		
	Excretion						
	Plasma clearance			Half-life (t1/2)			
Nimbaflavone	5.687			1.1			
Isonimocinolide	6.082			0.961			
Nicotiflorin	1.415			4.272			
	Toxicity						
	hERG blockers	Liver toxicity (DILI)	AMES mutagenicity	Acute oral toxicity	Skin sensitization	Genotoxicity	Neurotoxicity	Overall toxicity profile
Nimbaflavone	0.071	0.742	0.793	0.888	0.923	0.979	0.599	Poor
Isonimocinolide	0.019	0.869	0.609	0.889	0.96	0.997	0.175	Poor
Nicotiflorin	0.011	0.915	0.734	0.045	0.984	0.884	0.002	Poor

The distribution profiles of nimbaflavone, isonimocinolide and nicotiflorin were investigated in order to appraise their systemic disposition and tissue penetration dynamics. As shown in Table 5, nimbaflavone displayed the most favorable pharmacokinetic behavior which could be described as moderate volume of distribution and fairly stable plasma residence. Conversely, both isonimocinolide and nicotiflorin showed poor penetration into tissue and poor metabolic stability which are potentially limiting factors for their efficacy to the deep infection sites. None of the three compounds are predicted to cross the blood brain barrier, which arguably constitutes a limited central nervous system permeability and thus, at least to a degree, reduces the neurotoxicity, however, does not enable its use in central nervous system-related infections. Overall, these results highlight that nimbaflavone has the greatest therapeutic potential, yet all three of these phytochemicals require structural screening for enhanced pharmacokinetic stability to achieve efficacious therapeutic distribution.

The metabolic profiling of main lead compounds revealed large differences in their interactions with the cytochrome P450 (CYP) enzymatic network. AS shown in Table 5, nimbaflavone proved to be a potential substrate of CYP3A4 and a strong inhibitor of CYP2C9; proposing it as an increased potential for clinically relevant drug-drug interactions (DDIs) if used in combination with other agents metabolized through these pathways and an inhibitory potential on CYP1A2 was found to be negligible. On the contrary, isonimocinolide and nicotiflorin showed negligible or non-observed interaction with CYP isoforms tested, signaling better metabolic safety margin and consequences of lesser chances of adverse metabolic interactions. Accordingly, the inhibitory profile of nimbaflavone makes it a careful candidate in polypharmacy settings; however, isonimocinolide and nicotiflorin appear to be metabolically more stable and therefore better candidates for possible therapeutic development against *A. baumannii*.

The pharmacokinetic study of nimbaflavone, isonimocinolide and nicotiflorin revealed large differences in their kinetics of clearance and systemic half-life. As shown in Table 5, nimbaflavone and isonimocinolide displayed rapid clearance, leading to rather short elimination half-lives. These kinetic features suggest a rapid hepatic processing and renal elimination of drug from the systemic compartment and can therefore cause a need for more frequent dosing regimens to maintain therapeutic levels of drug concentration. In contrast, nicotiflorin displayed a significantly slow rate of clearance and half-life, indicating a slow systemic residence time. Such a sustained exposure leads one to believe that nicotiflorin may be endowed with a greater duration of action of the pharmacologic action of the two other compounds. Overall, although the rapid rate at which nimbaflavone and isonimocinolide are eliminated may limit the possibility of drug accumulation, the long half-life of nicotiflorin has unique advantage in terms of dose frequency when targeting *A. baumannii* infections.

A thorough *in silico* toxicological evaluation of nimbaflavone, isonimocinolide and nicotiflorin identified serious safety concerns, despite a relatively low predicted risk of cardiotoxicity and neurotoxicity. As shown in Table 5, all three compounds had high possibility of hepatotoxicity, mutagenicity and genotoxicity. In particular, nicotiflorin had the highest probability of drug-induced liver injury (DILI), while nimbaflavone and isonimocinolide showed high correlation with the genotoxic and mutagenic potential. Concerning acute systemic safety, predictions showed that nimbaflavone and isonimocinolide had high acute oral toxicity profile. Furthermore, each one of the phytochemicals was found to have the ability to cause skin sensitization, which suggests potential allergic or inflammatory response on exposure to the chemical. Whilst the lack of significant cardiac and neurological hazards can be considered a beneficial feature, the overall toxicological profiles are less than ideal. Consequently, these derivatives of *A. indica* should undergo a confidential matter of safety assessment and structure modifications to reduce hepatotoxic and genotoxic risks before further clinical development occurs.

The computational analysis of *A. indica* (Neem) reveals the phytoconstituents have significant antimicrobial activity against MDR pathogen (*A. baumannii*). Through molecular docking several constituents were found to have potent inhibition of the essential bacterial proteins with nimbaflavone showing most binding affinity especially towards the 5YDB target. Pharmacokinetic profiling done through ADMET screening resulted in a complex balance of efficacy and drug-likeness. Nimbaflavone is also shown to have moderate intestinal absorption and a stable systemic distribution whereas Nicotiflorin is linked with an extended elimination half-life and therefore the potential for long term therapeutic exposure. However, the oral bioavailability of these crude phytochemicals still has a considerable limitation. From a metabolic perspective, the compounds do not generally exhibit a particularly unfavorable safety profile, however, the inhibition of CYP2C9 by nimbaflavone raises awareness of the need to be vigilant for the occ-

urrence of possible drug-drug interactions in a polypharmacy setting.

Toxicological evaluation revealed considerable risks for hepatotoxicity and genotoxicity among the lead molecules and these become the major obstacles for clinical development. These results highlight an absolute need for thorough exercise of safety profiling before carrying any of these agents forward to a therapeutic application.

Discussion:

The escalating threat of AMR, particularly among opportunistic pathogens like *A. baumannii* (1), underscores the urgent need for alternative therapeutic strategies. This study aimed to investigate the *in-silico* inhibitory activity of A. *indica* phytochemicals against key proteins of *A. baumannii*. The molecular docking studies performed during this study showed that the chosen neem phytochemicals have good binding affinities ranging from -8.7 to -9.9 (kcal /mol). These values point to highly promising leads for inhibitors. Subsequent research by Mahmoud et al., (12) highlighted the latest progress in the computational drug discovery, especially the combination of molecular docking and ADMET screening, which can rapidly evaluate the pharmacokinetic properties of plant-deri ved compounds before experimental evaluation at a later stage, which is very costly.

The binding energies that are observed here are comparable to the studies that were published in the literature (12), and constituents of neem, which displayed similar affinities while tested against bacterial enzymes such as DNA gyrase and FtsZ, thereby suggesting that these components can be useful in the disruption of key functions in bacteria such as replication and cell division. Furthermore, the antimicrobial possibilities of neem do not end up in the computerized projections. As reported in a study by Owolabi et al., (13), significant zone of inhibition against different clinical and foodborne pathogens has been demonstrated by experimental investigations conducted with neem leaf extracts. These empirical observations are complimented by the study of Mudenda et al., (8) which confirmed the efficacy of Neem against diverse Gram-positive and Gram-negative bacteria and attributed these broad-spectrum activities to the synergies of the various phytochemicals present in the extract.

Despite the good docking scores indicative of potent inhibition, ADMET screening during this study demonstrated that there are significant pharmacokinetic limitations. Many of the phytochemicals which scored well had low oral bioavailability as well as potential toxicity. This agrees with the study by Owolabi et al., (11) who reported that many plant-derived compounds have failed to satisfy rigorous drug-likeness criteria in their native form without chemical modification. Therefore, although these phytochemicals demonstrate excellent *in silico* properties, there is still a need for empirical *in vitro* and *in vivo* validation.

This study is limited on the fact that it relies entirely on computational predictions based on no experimental validation, which raises the concern that the reported binding affinity for *A. baumannii* may not express itself as actual biological activity. Moreover, the computational predicted limitations such as the insufficient bioavailability and potential prospective toxicity require full *in vitro* and *in vivo* evaluations before consideration of therapeutic deployment.

Conclusion:

Azadirachta indica shows good *in-silico* potential activity against drug-resistant *A. baumannii* and some effective compounds including isonimocinolide, nicotiflorin and nimbaflavone were identified as high-affinity leads. Despite such promising scaffolds, the limitations of ADMET i. e. poor oral bioavailability and risk of hepatotoxicity/genotoxicity, emphasizes the importance of structural optimization. Further *in vitro* validation of these neem-derived compounds is mandatory to refine the pharmacokinetic profiles and the safety of these compounds in an effort to develop therapeutic material.

Prospective investigations should be focused on isolation and *in-vitro* validation of the identified lead entities in order to validate their inhibitory function against MDR *A. baumannii*. In view of known ADMET drawbacks, it is advised to pay attention to structural optimization and development of new approaches to drug formulation for the sake of higher bioavailability and safety. More so, *in-vivo* safety analyses, as well as studies investigating the synergistic interactions underlying the simplicity between these phytochemicals and standard antibiotics, are imperative to bypass existent resistance mechanisms.

Ethical statement:

This study was conducted entirely *in-silico*, using computational tools, and did not require human subjects, use animal subjects or clinical specimens, and was therefore not subject to the overview of local institutional ethics board scrutiny.

Contributions of authors:

PD supervised the study; IB conceived the study and was the main author in the research; and KP co-supervised the study. All authors read and approved the final manuscript submitted for publication.

Source of funding:

No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

Artificial Intelligence (AI) use disclosure:

Generative AI; ChatGPT and Claude AI, were used to assist in the interpretation and clarification of results. Stealthwriter AI and Humanize AI, were used in this study for paraphrasing and spell check. My bib was used to arrange the references. The authors reviewed, edited and validated all results to ensure accuracy and scientific integrity.

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Original Article **Open Access**

Sexual and reproductive health practices among fresh female graduates in Iseyin, Oyo State, Nigeria

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Abstract:

Background: Fresh graduates of tertiary institutions are at a stage in their lives characterized by searching, discovery, and sexual experimentation. They engage in high-risk sexual behaviours and poor reproductive health practices. The objective of this study is to assess the sexual and reproductive health practices of young female graduates and to highlight the factors associated with these practices.
Methodology: A cross-sectional study was conducted among 413 fresh female graduates from Nigerian tertiary institutions who were on the one-year National Youth Service Corps (NYSC) orientation camp at Iseyin, Oyo state. Data on sociodemographic characteristics, and sexual and reproductive health practices were collected using a semi-structured, self-administered pretested questionnaire. The data were analysed using SPSS version 22. Association between variables was determined using Chi square and Fisher Exact tests, with level of significance at $p<0.05$.
Results: The mean age of the 413 respondents was 25±2.2 years. More than half of the respondents (63.7%, n=263) had ever had sexual intercourse, with the youngest age at sexual debut being 14 years. About half of those who are sexually active (48.3%, n=127) did not use contraceptives. Condom was the commonest type of contraceptive used (79.4%, n=108/136). Multiple sexual partners (42%, n=110/263), poor contraceptive use (49%, n=129/263), early sexual debut (18.6%, n=49/263), pregnancy (34.6%, n=91/263), unwanted pregnancy (11.0%, n=29/263), history of abortion (17.1%, n=45/263) and induced abortion (14.8%, n=39/263) were recorded among sexually active respondents. Multiple sexual partners (OR=4.2, 95% CI=1.503-11.469, $p=0.006$) and age at sexual debut (OR=2.2, 95% CI=1.065-4.601 $p=0.033$) were significantly associated with history of abortion among the respondents, and multiple sexual partners was significantly associated with pregnancy (OR=7.2, 95% CI=2.743-20.118, $p=0.000$).
Conclusion: In this study, a high proportion of the study participants had risky sexual and poor reproductive health practices. Targeted reproductive health promotion and education interventions for this population set would be highly beneficial.

Keywords: Sexual health, behaviour, practices, fresh female graduates, abortions, multiple sexual partners

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Pratiques de santé sexuelle et reproductive chez les jeunes diplômées d'Iseyin, État d'Oyo, Nigéria

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Résumé:

Contexte: Les jeunes diplômées de l'enseignement supérieur traversent une période de recherche, de découverte et d'expérimentation sexuelle. Elles adoptent des comportements sexuels à risque et de mauvaises pratiques en matière de santé reproductive. L'objectif de cette étude est d'évaluer les pratiques en matière de santé sexuelle et reproductive de jeunes femmes diplômées et d'identifier les facteurs associés à ces pratiques.
Méthodologie: Une étude transversale a été menée auprès de 413 jeunes femmes fraîchement diplômées d'établissements d'enseignement supérieur nigérians, participant au camp d'orientation d'un an du Corps national de la jeunesse (NYSC) à Iseyin, dans l'État d'Oyo. Les données relatives aux caractéristiques sociodémographiques et aux pratiques en matière de santé sexuelle et reproductive ont été recueillies à l'aide d'un questionnaire semi-structuré, auto-administré et prétesté. Les données ont été analysées avec le logiciel SPSS version 22. L'association entre les variables a été déterminée par les tests du χ^2 et de Fisher, avec un seuil de signification fixé à $p<0,05$.
Résultats: L'âge moyen des 413 participantes était de 25±2,2 ans. Plus de la moitié d'entre elles (63,7%, n=263) avaient déjà eu des rapports sexuels, l'âge le plus jeune au premier rapport étant de 14 ans. Environ la moitié des personnes sexuellement actives (48,3%, n=127) n'utilisaient pas de contraception. Le préservatif était le moyen de contraception le plus fréquemment utilisé (79,4%, n=108/136). Parmi les répondants sexuellement actifs, on a relevé les facteurs suivants : partenaires sexuels multiples (42,0%, n=110/263), utilisation insuffisante de la contraception (49,0%, n=129/263), début précoce de l'activité sexuelle (18,6%, n=49/263), grossesse (34,6%, n=91/263), grossesse non désirée (11,0%, n=29/263), antécédents d'avortement (17,1%, n=45/263) et d'avortement provoqué (14,8%, n=39/263). Le fait d'avoir eu plusieurs partenaires sexuels (OR=4,2, IC à 95%=1,503-11,469, p=0,006) et l'âge au premier rapport sexuel (OR=2,2, IC à 95%=1,065-4,601, p=0,033) étaient significativement associés aux antécédents d'avortement chez les participantes. De plus, le fait d'avoir eu plusieurs partenaires sexuels était significativement associé à la grossesse (OR=7,2, IC à 95%=2,743-20,118, p=0,000).
Conclusion: Dans cette étude, une forte proportion des participantes présentaient des comportements sexuels à risque et de mauvaises pratiques en matière de santé reproductive. Des interventions ciblées de promotion et d'éducation à la santé reproductive auprès de cette population seraient très bénéfiques.

Mots-clés: Santé sexuelle, comportements, pratiques, jeunes diplômées, avortements, partenaires sexuels multiples

Introduction:

Sexual behaviour is a core aspect of human life shaped by cultural, social, and personal factors (1,2). Poverty, urbanization, conflicting sexual values, and premarital sexual activity contribute to early sexual initiation among youths (3,4). Media glamorization of sex, globalization of communication, and declining traditional norms influence adolescent lifestyle, leading to earlier sexual debut and increased sexual activities, including multiple partners and short-term unions (5)

Tertiary students, free from parental and school restrictions, often engage in risky sexual behaviours (6). These include unprotected sex, early sexual debut, substance use before sex, multiple partners, and transactional sex, which increase the risk of contracting sexually transmitted infections including HIV (7). Many youths lack awareness of the consequences while their sexual and reproductive health needs remain largely unmet (8).

Fresh graduates of tertiary institutions are at a stage in their lives characterized by searching, discovery, and sexual experimentation (9). They live and socialize with large numbers of other young adults, which encourages sexual activities that are not mutually monogamous. Despite differences by age or location, contraceptive use in sub-Saharan Africa remains low due to concerns about side effects, lack of resources, opposition, poor awareness, and gender power issues (10,11).

Poor condom use is often linked to lack of sexual communication, requiring more research on partner dialogue (12,13). In Ethiopia, only 4.9% of university students used contraceptives, mainly pills (44%) and injectables (13%) (14). Unplanned pregnancies are widespread and often result in unsafe abortions due to restrictive laws (15-17), contributing to 13% of global maternal deaths (16). Most women who abort are under 30 years of age and post-secondary educated (14). Unplanned pregnancy is the main reason for abortion, with nearly half opting for unsafe methods (18), while youth aged 15–24 years account for over 50% of abortion-related deaths in Africa (19).

One-fifth of the Nigeria's 200 million population is made of youth within the age group 15–24 years. The growing numbers and poor reproductive health are serious concerns. Limited access to services leads to high rates of unwanted pregnancies, abortions, HIV/AIDS, and related issues. This study examined sexual and reproductive health practices and associated factors among fresh female graduates during NYSC orientation in Iseyin, Oyo State, Nigeria.

Materials and method:

Study area:

The study was conducted at the NYSC camp in Iseyin, Oyo State, southwest zone, Nigeria. The NYSC scheme promotes youth development, nation-building, and self-reliance, admitting two batches ("A" and "B") yearly at the time of data collection. The three-week orientation camp offers opportunities for research and counselling.

Ethical consideration:

Ethical approval was obtained from the Oyo State Ministry of Health. Permission was granted by the NYSC State Coordinator and Camp Commandant. Informed consent was obtained from each participant. A free seminar on using Reproductive Life Plan (RLP) to set reproductive goals followed the survey, with investigators addressing questions.

Study population:

The study population consisted of fresh female graduates from Nigerian universities (degree holders) and polytechnics (HND holders) who were attending the NYSC orientation program scheme.

Study design:

This cross-sectional study was part of larger research on reproductive life plans, perception of preconception care, and sexual and reproductive health practices among fresh female NYSC graduates in Southwest Nigeria.

Sampling technique:

A simple random sampling technique was used to select eligible female graduates at the NYSC orientation camp. A sampling frame comprising all eligible female graduates present at the camp was obtained, and participants were selected randomly from the sampling frame. The study was explained to the selected participants, and those who provided informed consent were recruited into the study.

Study instrument:

A semi-structured, self-administered questionnaire pretested on a previous batch of Corp members was used to collect data on respondents' socio-demographics, age at sexual debut, history of multiple sexual partners, pregnancy history, abortion, and contraceptive use. It was administered in English.

Data collection:

Two resident doctors were trained in research ethics and recruited to distribute and collect questionnaires. After obtaining informed consent, they administered the surveys to female corps members. The investigators supervised all aspects of data collection at the orientation camp.

Data analysis:

Data were analyzed using SPSS version 22 (Chicago, Illinois). Results were presented in tables, charts, and graphs. Chi-square test was used to determine associations between categorical variables at a 5% significance level. Logistic regression was used to calculate odds ratios and confidence intervals.

Results:

Sociodemographic characteristics of the study participants:

A total of 413 female graduates were selected for the study. The mean age of the respondents who participated in this study was 25±2.2 years, with about 60.0% being 25 years and above, while 40.0% were within the age range of 18-24 years. The majority of the respondents (74.1%) had a university degree. About 54.5% of the respondents hailed from the Yoruba ethnicity group, with the majority (80.6%) being single.

Sexual health behaviour and practices of the respondents:

Table 2 shows that 63.7% (n=263) of the participants had ever had sex, and 78.7% (n=207) within the past year. Most (71.5%, n=188) had their first intercourse at 19–24 years, 51.0% (n=134) used contraceptives at debut. The youngest age of sexual debut was 14 years. Fig 1 above shows the frequency distribution of contraceptive types used by the 263 female respondents at 1st sexual intercourse.

Table1: Sociodemographic characteristics of fresh university graduate respondents in Iseyin, Oyo State, Nigeria

Characteristics	Frequency	Percentage (%)
Type of tertiary institution attended		
University	306	74.1
Polytechnic	105	25.4
Others	2	0.5
Degree attained		
B.Sc.	243	58.8
B.A.	39	9.4
HND	105	25.4
Others	26	6.3
Age at last birthday (in years)		
18-24	165	40.0
≥25	248	60.0
Religion		
Christianity	351	85.0
Islam	62	15.0
Ethnicity		
Yoruba	225	54.5
Igbo	104	25.2
Hausa	15	3.6
Others	69	16.7
Marital status		
Single	333	80.6
Married	80	19.4

B.Sc. = Bachelor of Science; B.A. = Bachelor of Arts; HND = Higher National Diploma; Others = College of Education and School of Nursing.

Table 2: Sexual health practices of fresh female university graduate respondents in Iseyin, Oyo State, Nigeria

Sexual practices	Frequency	Percentage
Ever had sexual intercourse (n=413)		
Yes	263	63.7
No	150	36.3
Had sexual intercourse in the last one year (n=263)		
Yes	207	78.7
No	56	21.3
Age at first sexual intercourse (in years) (n=263)		
≤18	49	18.6
19-24	188	71.5
≥25	26	9.9
Use of contraceptive at first sexual intercourse (n=263)		
Yes	134	51.0
No	129	49.0
Number of sexual partners (n=263)		
1	153	58.0
>1	110	42.0
History of pregnancy (n=263)		
Yes	91	34.6
No	172	65.4

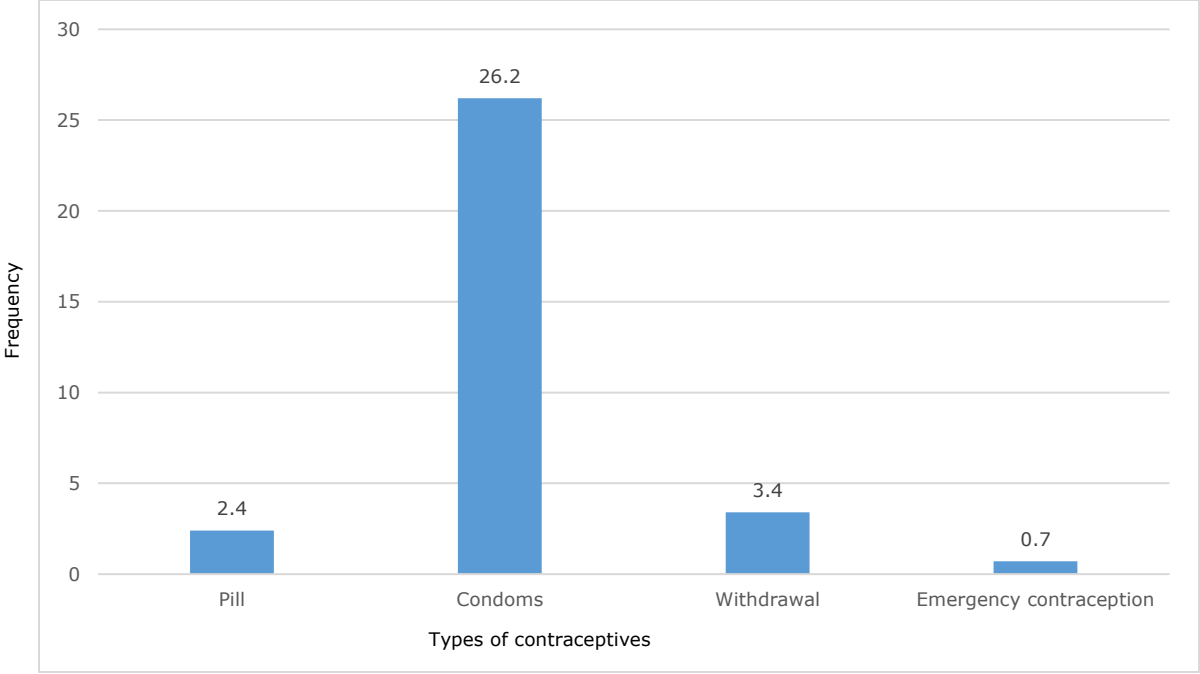

Fig 1: Types of contraceptives used among female fresh university graduates in Iseyin, Oyo State, Nigeria

Table 3 shows that a few respondents (n=45) had experienced abortion. Among these, 86.7% (n=39) were induced abortions and 22.3% (n=6) were spontaneous abortions. Of the 45 cases, 64.4% cited unpreparedness for pregnancy or motherhood as the reason for procuring abortion, 17.8% feared consequences of pregnancy, 4.4% expected parental rejection, and 2.2% gave no reason for procuring abortion.

Table 3: Frequency distribution of fresh female university graduate respondents in Iseyin, Nigeria, with respect to history and experience of abortion

Variables	Frequency	Percentage
Ever aborted a pregnancy (n=263)		
Yes	45	17.1
No	218	82.9
Number of induced abortion (n=39)		
1	26	57.8
2	11	24.4
3	2	4.4
	6	13.3
Number of spontaneous abortion (n=6)		
1	4	8.8
2	2	4.4
Reasons for abortion (n=45)		
Unprepared (not ready) for responsibility	29	64.4
Afraid of the consequences of keeping the baby	8	17.8
Parental refusal of marriage	2	4.4
Health reason	1	2.2
Rape	1	2.2
No reason	1	2.2
Others	3	6.7

Factors associated with sexual risk behaviours among the respondents:

Age at sexual debut was significantly associated with only abortion (x^2=7.940, p= 0.0189), with respondents with age of sexual debut ≤ 18 years having significantly higher frequency of abortion (28.6%) while respondents with age of sexual debut >25 years had significantly lower frequency of abortion (3.8%) (Table 4). However, age at sexual debut was not significantly associated with contraceptive use (x^2=2.591, p=0.6284) or history of pregnancy (x^2=2.510, p=0.2850).

Table 5 shows that respondents who had been pregnant were four times more likely to have more than one (multiple) sexual partners, while those with abortion history were seven times more likely to have multiple sexual partners than those without abortion history. Table 6 shows that respondents who are 18 years and below are twice likely to have history of abortion (28.6%) compared to those 18 years and above (15.5%) (OR=2.2, 95% CI=1.065-4.601, p=0.033)

Table 4: Bivariate analysis of age at sexual debut with contraceptives usage, history of pregnancy and abortion among fresh female graduate respondents in Iseyin, Oyo State, Nigeria

Variables	Age at sexual debut (years)			Total (n=263)	χ^2	*p*-value
	≤18 (n=49)	19-24 (n=188)	≥25 (n=26)			
Contraceptives use						
Condoms	18 (36.7)	82 (43.6)	8 (30.8)	108 (41.1)	2.591	0.6284
Others	7 (14.3)	18 (9.6)	3 (11.5)	28 (10.6)		
No contraceptive use	24 (49.0)	88 (46.8)	15 (57.7)	127 (48.3)		
Ever been pregnant						
Yes	19 (38.8)	60 (31.9)	12 (46.2)	91 (34.6)	2.510	0.2850
No	30 (61.2)	128 (68.1)	14 (53.8)	172 (65.4)		
Ever had abortion						
Yes	14 (28.6)	30 (16.0)	1 (3.8)	45 (17.1)	7.940	0.0189*
No	35 (71.4)	158 (84.0)	25 (96.2)	218 (82.9)		

*Statistically significant at p<0.05

Table 5: Bivariate analysis of number of sexual partners with history of pregnancy and abortion among fresh female university graduate respondents in Iseyin, Oyo State, Nigeria

Variable	No of sexual partners		*p*-value (OR) CI
	One (n, %)	More than one (n, %)	
Ever been pregnant			
Yes	79 (32.5)	12 (66.7)	0.006* (4.2) 1.503-11.469
No (ref)	164 (67.5)	8 (33.3)	
Ever aborted			
Yes	35 (14.3)	10 (55.6)	0.000* (7.4) 2.743-20.118
No (ref)	210 (85.7)	8 (44.4)	

Table 6: Bivariate analysis of age at sexual debut and history of abortion among fresh female university graduate respondents in Iseyin, Oyo State, Nigeria

Variable	Ever aborted		*p*-value (OR) CI
	Yes (n, %)	No (n, %)	
Age of sexual debut			
18 years and younger (ref)	15 (33.3)	52 (23.9)	0.033* (2.2) 1.065-4.601
Above 18 years	30 (77.7)	166 (76.1)	
Total	**45**	**218**	

Discussion:

This study assessed the sexual and reproductive health practices of fresh female graduates. The mean respondent age was 25±2.2 years, with 40% aged 18–24 years and this agrees with the study by Obembe et al., in 2017 (20), who reported a similar mean age of 18.26 ± 3.45 among students. A review by Cooper et al., in 2007 (7) found a sexual debut age of 17 years and a median of two partners, while another review by Hoque et al., in 2011 (21) found debut at 19 years with 2–4 partners, comparable to the youngest debut age of 14 years in our study. Findings from our study show that most respondents were Yoruba and single, similar to findings of previous studies (20,22).

Sexual activity was reported by 63.7% of the respondents, with 42% having multiple partners. A survey of female undergraduate students in two Nigerian universities reported that 47.6% were sexually active, with the same percentage having had sex in the past year, and 61.4% engaging in unprotected sex (18). Over half of the participants (51.7%) in this study reported contraceptive use, primarily condoms (41.1%). A previous study reported that 44.6% of participants were confident in the use of female condoms (20). Another study reported 38.6% of participants used condoms, 26.1% emergency contraception, and 63.1% were aware of prevention drugs (18). A study from Ethiopia reported 10% contraceptive use, while 30.1% usage was reported by Oyedokun et al., in 2007 (23).

Our finding of 48.3% respondents with practice of unprotected sex (49%) is comparatively lower than those from South Africa (18, 24) which reported 60.7% among female undergraduates, and far lower than 86.3% reported in Ethiopia (14). About 17% of the respondents in our study had ever had an abortion, which is lower than 24.1% reported among hairdressers, and 33% among female undergraduates in Benin (25), while findings from Cadmus and Owoaje in 2011 (19) reported 93.3% among 30 sexually active students.

Abortion is common among young unmarried women due to stigma of unwanted pregnancy (26). A study reported that over half of respondents would consider abortion if faced with an unwanted pregnancy (22). Among those who had abortions in our study, 86.7% were induced and 13.3% were spontaneous abortions which is comparably higher than 66.7% induced, and 33.5% multiple abortions in the study by Olukoya et al., (27). In our study, reasons for abortions included unpreparedness, fear of academic disruption, and age, which is in line with findings of Cadmus and Owoaje in 2011 (19).

Bivariate analysis showed that respondents with multiple sexual partners had fourfold likelihood of being pregnant (OR=4.2, 96% CI =1.503-11.469, p=0.006) which contrasts the finding of Omokhodion and Balogun (28). The likelihood for abortion history for respondents with multiple sexual partners increased sevenfold (OR = 7.4, 95% CI = 2.743-20.118, p = 0.000) consistent with report of Alemayehu et al., in 2017 (29). Respondents who are 18 years and below are twice likely to have history of abortion (28.6%) compared to those 18 years and above (15.5%) (OR=2.2, 95% CI=1.065-4.601, p=0.033)., which is contradicts what was reported by Onukwugha in his review of the NDHS 2003-2018, where he reported that higher rates were found in women aged 20–24 are more likely to have aborted compared to the younger age group Onukwugha et al., in 2020 (30).

Our study has some limitations. First, the cross-sectional design limits the ability to establish causal relationships between the identified factors and sexual and reproductive health outcomes. Secondly, data on sexual behaviours, contraceptive use, pregnancy, and abortion were self-reported and involved sensitive issues; therefore, recall and social desirability biases may have resulted in underreporting or inaccurate reporting of some practices. Nevertheless, our study showed that many of the fresh female graduates engaged in risky sexual behaviours marked by early sexual debut, multiple sexual partners, poor contraceptive use, and relatively high abortion rate.

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Contributions of authors:

FOO conceived and designed the study, developed the study protocol and data collection instrument, contributed and supervised data collection, reviewed the analysis and interpretation of findings. OCO drafted the manuscript, contributed to the study design and methodology, participated in data collection and data management, analyzed and interpreted the data. AMA contributed to the study design and methodology, participated in data collection, reviewed the analysis and interpretation of findings. All authors reviewed and approved the

final draft of the manuscript and agreed to be accountable for all aspects of the work.

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Conflict of interests:

Authors report no financial and non-financial conflicting interests in the reporting and writing of this research.

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Short Communication **Open Access**

Multidrug-resistant *Staphylococcus aureus* associated with neonatal sepsis in the special care baby unit of a secondary healthcare facility in Katsina State, Nigeria

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Abstract:

Background: Neonatal sepsis remains a major cause of morbidity and mortality in developing countries, with *Staphylococcus aureus* being one of the leading causative organisms. The rising occurrence of multidrug-resistant (MDR) strains poses significant therapeutic challenges. This study aimed to determine the antibiotic resistance pattern and multiple antibiotic resistance (MAR) index of *S. aureus* isolated from neonatal sepsis cases in the Special Care Baby Unit (SCBU) of a secondary healthcare facility in Katsina State, Nigeria.
Methodology: A hospital-based cross-sectional study was conducted involving 155 neonates with suspected sepsis. Blood samples were aseptically collected and cultured using standard microbiological techniques. *Staphylococcus aureus* isolates were identified through colony morphology, Gram staining, and biochemical tests. Antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines. Multi-drug resistance was defined as resistance of an isolate to three or more classes of antibiotics. The MAR index for each isolate was calculated as the ratio of the number of antibiotics resisted to the total number tested.
Results: Of the blood samples analyzed for 155 clinical neonatal sepsis cases, 65 (41.9%) were culture-positive for bacterial pathogens, with 31 (20.0%) *S. aureus* isolates, which exhibited high resistance to erythromycin (87.0%), cefoxitin (83.0%), ampicillin (64.0%) and gentamicin (64.0%). Although resistance to imipenem was lowest at 51.0% among the antibiotics tested, this value still indicated a relatively high resistance rate. Overall, MDR was detected in 74.0% of the isolates, indicating high AMR burden among *S. aureus* associated with neonatal sepsis. Among the isolates, 83.9% were methicillin-resistant *S. aureus* (MRSA) while 64.5% were gentamicin-resistant *S. aureus* (GRSA). The MAR index of the *S. aureus* isolates ranged from 0.2 to 1.0, with a mean value of 0.7±0.3, but most isolates (93.5%) exhibited MAR index values higher than 0.2.
Conclusion: The high prevalence of MDR and high MAR index values among *S. aureus* isolates indicate significant antibiotic selection pressure within the SCBU of the facility studied. Strengthened antimicrobial stewardship and routine AMR surveillance are urgently required to improve neonatal sepsis outcomes.

Keywords: Neonatal sepsis, *Staphylococcus aureus*, Multiple antibiotic resistance index, Multidrug resistance, SCBU.

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Staphylococcus aureus multirésistant associé à une septicémie néonatale dans l'unité de soins intensifs néonatals d'un établissement de soins de santé secondaires de l'État de Katsina, au Nigéria

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Résumé:

Contexte: La septicémie néonatale demeure une cause majeure de morbidité et de mortalité dans les pays en développement, *Staphylococcus aureus* étant l'un des principaux agents pathogènes. L'augmentation de la fréquence des souches multirésistantes (MDR) pose d'importants défis thérapeutiques. Cette étude visait à déterminer le profil de résistance aux antibiotiques et l'indice de multirésistance aux antibiotiques (MAR) de *S. aureus* isolé chez des nouveau-nés atteints de sepsis dans l'unité de soins intensifs néonatals (USIN) d'un établissement de soins de santé secondaires de l'État de Katsina, au Nigéria.
Méthodologie: Une étude transversale hospitalière a été menée auprès de 155 nouveau-nés présentant une suspicion de sepsis. Des échantillons de sang ont été prélevés de manière aseptique et mis en culture selon les techniques microbiologiques standard. Les isolats de *S. aureus* ont été identifiés par morphologie des colonies, coloration de Gram et tests biochimiques. L'antibiogramme a été réalisé par la méthode de diffusion sur disque de Kirby-Bauer, conformément aux recommandations d'Institut des Normes de Laboratoire Clinique (CLSI). La multirésistance a été définie comme la résistance d'un isolat à trois classes d'antibiotiques ou plus. L'indice MAR de chaque isolat a été calculé comme le rapport entre le nombre d'antibiotiques auxquels il est résistant et le nombre total d'antibiotiques testés.
Résultats: Sur 155 échantillons sanguins analysés pour des cas cliniques de sepsis néonatal, 65 (41,9 %) étaient positifs à la culture pour des agents pathogènes bactériens, dont 31 (20,0 %) isolats de *S. aureus*. Ces isolats présentaient une forte résistance à l'érythromycine (87,0%), à la céfoxitine (83,0%), à l'ampicilline (64,0%) et à la gentamicine (64,0%). Bien que la résistance à l'imipénem soit la plus faible (51,0%) parmi les antibiotiques testés, ce taux reste relativement élevé. Au total, une multirésistance a été détectée chez 74,0% des isolats, témoignant d'une forte prévalence de résistance aux antimicrobiens chez *S. aureus* associé au sepsis néonatal. Parmi les isolats, 83,9 % étaient des *S. aureus* résistants à la méthicilline (SARM) et 64,5 % des *S. aureus* résistants à la gentamicine (SARM). L'indice MAR des isolats de *S. aureus* variait de 0,2 à 1,0, avec une valeur moyenne de 0,7±0,3. Cependant, la plupart des isolats (93,5%) présentaient des valeurs d'indice MAR supérieures à 0,2.
Conclusion: La forte prévalence de la multirésistance et les valeurs élevées de l'indice MAR parmi les isolats de *S. aureus* indiquent une pression de sélection antibiotique importante au sein de l'unité de soins intensifs néonatals (USIN) de l'établissement étudié. Un renforcement de la gestion des antimicrobiens et une surveillance systématique de la résistance aux antimicrobiens sont nécessaires de toute urgence pour améliorer la prise en charge de la septicémie néonatale.

Mots-clés: Septicémie néonatale, *Staphylococcus aureus*, Indice de résistance à de multiples antibiotiques, Multirésistance, USIN.

Introduction:

Worldwide, neonatal sepsis is a leading cause of morbidity and mortality, particularly in low- and middle-income countries with limited access to prompt diagnosis and effective antibiotic treatment (1). Bacterial infections continue to be the primary cause of sepsis in this age group, and neonates with sepsis do not normally present specific clinical signs and symptoms. This lack of specific clinical presentations makes early diagnosis and treatment difficult (2).

Staphylococcus aureus remains one of the most common bacterial pathogens associated with bloodstream infections in neonates (3). More than half of the culture-confirmed neonatal sepsis cases in a study in southwest Nigeria were caused by *S. aureus*, and the isolates had a high frequency of resistance to methicillin (4). Similarly, *S. aureus* has been identified by regional surveillance studies as a major cause of neonatal infections, often associated with high levels of antibiotic resistance (5). This is because *S. aureus* has a remarkable ability to easily develop resistance by horizontal gene transfer, mutation of the chromosome, or selective pressure arising from excessive antibiotic usage (6). The growing prevalence of antibiotic-resistant bacteria in neonatal care settings poses a great threat to effective clinical management. The World Health Organization considers antibiotic resistance a global threat that causes approximately 700,000 deaths worldwide, rendering antibiotics ineffective against microorganisms (7).

The high rate of multidrug resistance (MDR) in *S. aureus* complicates empirical treatment protocols and is linked to higher mortality, longer hospital stays, and higher health-

care costs (8). (9) opined that antibiotic misuse and a lack of antimicrobial stewardship infrastructure in Nigeria and other sub-Saharan African countries contribute to the burden of antimicrobial resistance in bloodstream isolates, thereby helping resistant strains persist and spread further. Although several studies have reported antimicrobial resistance patterns of *S. aureus* in Nigeria, updated regional data, particularly in special care baby units (SCBUs) are lacking due to the high risk of antibiotic exposure.

Antibiotic resistance assessment at the isolate level is essential for directing empirical treatment and infection control measures (3). High multiple antibiotic resistance (MAR) indices indicate greater exposure to antibiotics, stronger selection pressure, and a higher risk of treatment failure. The MAR index is a numerical indicator that is determined for each isolate based on the number of antibiotics to which it is resistant relative to the total number of antibiotics tested (10). Organisms with a high MAR index rate exhibit unsuccessful antibiotic treatments and a high source of potentially dangerous drug-resistant bacteria making continuous monitoring necessary (7).

Despite its clinical significance, there is a paucity of data on MAR indices of *S. aureus* isolates from neonatal sepsis cases in Katsina, Nigeria. The study, therefore, aims to determine the pattern of antibiotic resistance and MAR indices of *S. aureus* in the SCBU of a secondary healthcare facility in Katsina State, and to assess the relationship between MRSA and MDR among the isolates, in order to better inform local antibiotic resistance policy and stewardship interventions.

Materials and method:

Study design and setting:

This study was a cross-sectional, hospital-based design carried out in the SCBU of Turai Yar'adua Maternity and Children Hospital, Katsina. The facility is located in Katsina metropolis, Katsina State, Nigeria, and is dedicated to offering specialized healthcare services to pregnant women, newborns, and children.

Ethical approval:

Ethical approval was obtained from the Katsina State Ministry of Health (MOH/ADM/SUB/1152/1/1160) based on the study compliance with ethical standards. Additionally, written informed consent was obtained from each neonate's parents/guardians, anonymity, and confidentiality were maintained.

Study population and sample size:

The study considered neonates with clinical signs and symptoms suggestive of neonatal sepsis according to International Management of Neonatal and Childhood Illnesses (IMNCHI) (11). The sample size was determined using the single population proportion formula; $n=Z^2p(1-p)/d^2$, and neonatal sepsis prevalence of 11.5% (p=0.115) reported in a previous study in Bangladesh (12) was used, with assumption of 5% error margin, a confidence interval 95%, and non-response rate of 5%. This gave a sample size of approximately 155 participants.

Selection criteria:

The inclusion criteria were neonates (0-28 days) admitted to the SCBU of the selected hospitals within the study period and symptomatic of neonatal sepsis according to the established clinical and haematological criteria of IMNCHI, any one or combinations of the symptoms and signs; poor feeding, convulsion, respiratory distress, hypothermia, hyperthermia, movement only when stimulated, and severe chest-in drawing (11), and whose parents granted permission for their neonates to participate in the study. Neonates admitted for non-infectious or surgical reasons, those with incomplete data, and those whose parents or legal guardians did not grant permission were excluded from the study.

Sampling technique:

The sampling technique for selection of participants was based on a similar study (13), which employs consecutive sampling technique. All neonates admitted to the SCBU who met the inclusion criteria were recruited consecutively as they presented during the study period.

Sample collection:

Blood specimens (2–3 ml) were collected aseptically by trained clinicians from neonates suspected of having sepsis prior to initiation of antimicrobial therapy, following standard clinical microbiology practices. Blood was immediately inoculated into conventional blood culture bottles. The needle was inserted through the rubber liner of the bottle cap, and the blood was dispensed into the culture medium bottle containing 10ml of Brain Heart Infusion (BHI) broth. Bottles were appropriately labeled with the patient's name and number, the date

and time of collection, and incubated aerobically at 37°C for 24 hours (2).

Isolation and identification of *S. aureus*:

Positive blood cultures were sub-cultured on Blood agar and Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hours. Pure colonies showing morphological characteristics consistent with *S. aureus* (golden yellow colonies on MSA and β-hemolysis on blood agar) were selected for further identification. Gram staining was performed to confirm Gram-positive cocci in clusters. Biochemical tests, including catalase and coagulase, were used to differentiate *S. aureus* from other staphylococcal species, in accordance with standard microbiological identification protocols (14).

Antibiotic susceptibility testing (AST):

The Kirby-Bauer disc diffusion technique was used for antibiotic susceptibility testing (AST) by inoculating 0.5 McFarland standard suspension of overnight cultures of *S. aureus* onto the surface of a sterile Mueller-Hinton agar plate. Commercial antibiotic disks were placed on the agar plate with a sterile forcep. The disks used were ampicillin (10µg), cefoxitin (30µg), ciprofloxacin (5µg), imipenem (10µg), erythromycin (15µg), and gentamicin (10µg). Following a 24-hour incubation at 37°C, the zone of inhibition was measured to the nearest millimeter and interpreted as susceptible (S), intermediate (I), or resistant (R) based on CLSI guidelines (15).

MRSA isolates were phenotypically identified if the cefoxitin disk zone of inhibition was less than or equal to 21mm, while isolates with zone of inhibition equal to 22 mm or higher were considered methicillin-susceptible *S. aureus* (MSSA) (15). Gentamicin resistant *S. aureus* (GRSA) was identified as isolates showing gentamicin disk zones of inhibition less than or equal to 12 mm (15). Multidrug resistance (MDR) was reported as resistance of a *S. aureus* isolate to three or more classes of antibiotics tested against the isolate (16).

Multiple antibiotic resistance (MAR) index:

The AST results were used to calculate the MAR index. MAR index was calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics tested for each *S. aureus* isolate. MARI=a/b, where 'a' is number of antibiotics the isolate is resistant to, and 'b' is total number of antibiotics tested. Isolate with MARI of ≥ 0.2 originates from a high-risk contamination source where several antibiotics are used.

Statistical analysis:

Data were analyzed using SPSS version 23.0. Descriptive statistics were used to summarize the data using averages, means, percentages, and standard deviations. Chi-square test was used to determine the relationship between MRSA and MDR. A p-value < 0.05 was considered statistically significant.

Results:

Distribution of *S. aureus* isolates in the study participants:

A total of 65 (41.9%) bacterial isolates were obtained from blood samples of 155 neonates with clinical suspicion of sepsis, of which 31 (20.0%) *S. aureus* isolates were identified biochemically as shown in Table 1.

Table 1: Prevalence of *Staphylococcus aureus* isolates from blood samples of neonates with neonatal sepsis

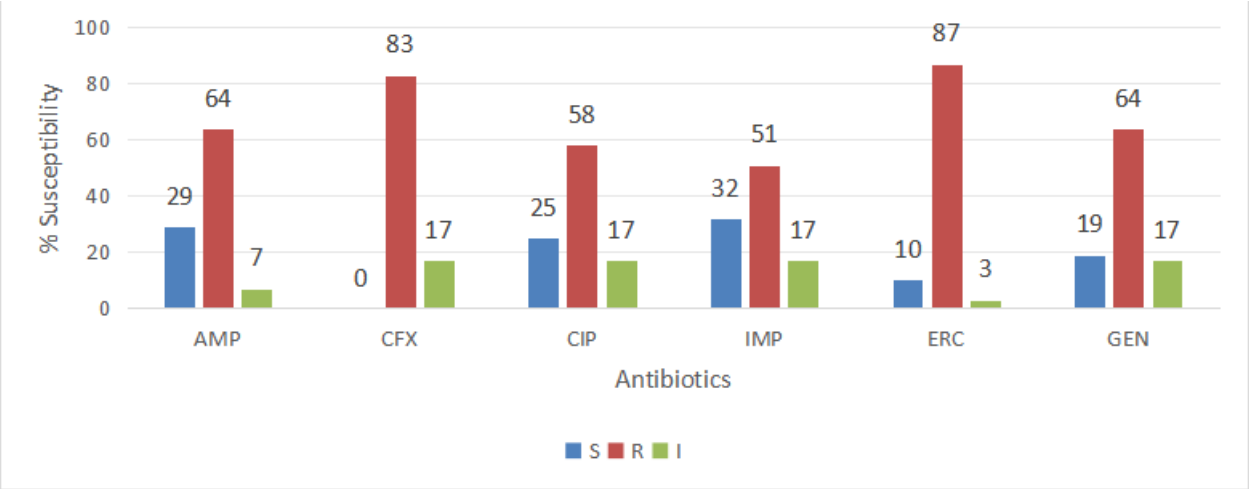
Participant blood samples	Number	Percentage
Negative cultures	90	59.1
Positive cultures	65	41.9
Staphylococcus aureus	31	20.0
Other pathogens	34	21.9

Antibiotic resistance of the *S. aureus* isolates:

Staphylococcus aureus isolates exhibited resistance to multiple classes of antibiotics (Fig 1). The highest rates of resistance were observed for erythromycin (87.0%) and ciprofloxacin (58.0%). Resistance was also high for ampicillin (64.0%) and gentamicin (64.0%). The highest susceptibility among the isolates was observed for imipenem (32.0%), although this value still reflects relatively low susceptibility overall. Approximately 50.0% of the isolates were resistant to more than half of the antibiotics tested.

Prevalence of methicillin, gentamicin and multidrug resistance among *S. aureus* isolates:

Table 2 shows the prevalence of methicillin gentamicin and multidrug resistance among the 31 *S. aureus* isolates. Of the isolates, 26 (83.9%) were identified as MRSA, while 5 (16.1%) were MSSA. Twenty isolates (64.5%) were resistant to gentamicin, while 11 (35.5%) were susceptible. Furthermore, 23 (74.0%) of the isolates were MDR, while 8 (26.0%) were non-MDR.



S= Sensitive; I= Intermediate; R= Resistance; AMP=Ampicillin CFX=Cefoxitin CIP=Ciprofloxacin IMP=Imipenem ERC=Erythromycin GEN=Gentamicin

Fig 1: Antibiotic susceptibility of *Staphylococcus isolates* to selected antibiotics

Table 2: Prevalence of methicillin, gentamicin and multidrug resistance among *Staphylococcus aureus* isolates.

Isolate resistance characteristics		**Frequency (n)**	**Percentage (%)**
Methicillin	MRSA	26	83.9
	MSSA	5	16.1
Gentamicin	GRSA	20	64.5
	GSSA	11	35.5
Multidrug	MDR	23	74.0
	Non-MDR	8	26.0

MSSA: Methicillin sensitive *Staphylococcus aureus*, MRSA: Methicillin resistant *Staphylococcus aureus,* GSSA: Gentamicin sensitive *Staphylococcus aureus*, GRSA: Gentamicin resistant *Staphylococcus aureus,* MDR: Multi-drug resistance

Distribution of multidrug-resistant *S. aureus* according to the number of antibiotics resisted:

Out of the 23 MDR isolated, 6 (26%), were resistant to three agents out of the six classes tested, 6 (26%) were resistant to five classes, 3 (13.0%) were resistant to four classes while 8 (35.0%) were resistant to all six agents (Table 3).

Multiple antibiotic resistance index:

In Fig 2, none of the *S. aureus* isolates had MAR index less than 0.2. The isolates with MARI of 0.2 were 2 (6.45%) while 28 out of 31 (93.5%) had MARI > 0.2. The overall mean MARI value for was 0.7±0.3.

Table 3: Distribution of multidrug-resistant S. *aureus* according to the number of antibiotics resisted

No of antibiotics	**MDR pattern**	**No of *S. aureus* isolates**	**Percentage (%)**
3	AMP, CIP, GEN	6	26.0
4	CFX, IMP, ERY, GEN	3	13.0
5	AMP, CFX, CIF, ERY, GEN	6	26.0
6	AMP, CFX, CIP, IMP, ERC, GEN	8	35.0
Total		**23**	**100.0**

AMP=Ampicillin, CFX=Cefoxitin, CIP=Ciprofloxacin, IMP=Imipenem, ERC= Erythromycin, GEN=Gentamicin

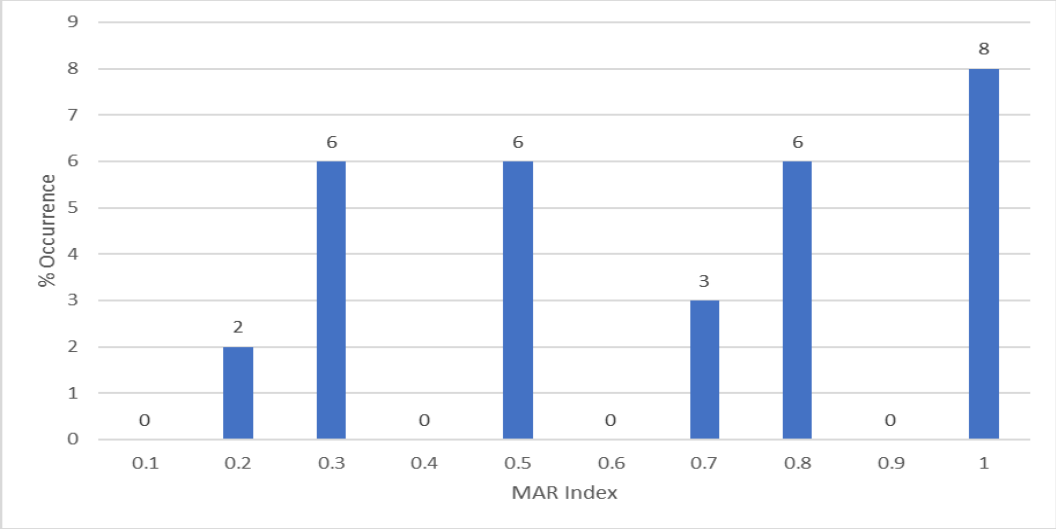


Fig 2: Frequency distribution of the multiple antibiotic-resistant indices of *Staphylococcus aureus* isolates

Table 4: Association between methicillin and multi-drug resistance among *Staphylococcus aureus* isolates

Bacteria	MDR n (%)	Non-MDR n (%)	Total	χ^2	*p* value
MRSA	19 (73.0)	7 (27.0)	26	0.105	0.746
MSSA	4 (80.0)	1 (20.0)	5		
Total	**23 (74.2)**	**8 (25.8)**	**31 (100.0)**		

MSSA=Methicillin sensitive *Staphylococcus aureus*; MRSA= Methicillin resistant *Staphylococcus aureus;* MDR=Multidrug resistance

Association of methicillin and multidrug resistance in *S. aureus* isolates:

No statistically significant association was observed between methicillin and multidrug resistance among the *S. aureus* isolates ($p > 0.05$) (Table 4).

Discussion:

This study evaluated the antibiotic resistance patterns and the MAR index of *S. aureus* isolated from neonatal sepsis cases in a special care baby unit (SCBU) of Turai Yar'adua Maternity and Children's Hospital, Katsina, Nigeria. The findings show high prevalence of AMR, MDR and high MAR index among *S. aureus* isolates of neonatal sepsis, signaling significant antibiotic selection pressure in the hospital environment.

The prevalence of *S. aureus* among culture-positive neonatal sepsis cases in this study is 20.0%, making it the most common bacterial isolate. This is consistent with reports identifying the organism as a major etiological agent of neonatal bloodstream infections in Nigeria and other low- and middle-income settings. A hospital-based study in north-central Nigeria reported *S. aureus* as the most prevalent organism (13). Another study in south-western Nigeria reported *S. aureus* as the most common isolated organism (2). In India, *S. aureus* was also the most commonly isolated pathogen (17). However, this is in contrast to the report of the study by Olorukooba et al., (18), in which Gram-negative bacteria (typically *Escherichia coli*) was the predominant organism. The discrepancy in microbial types in neonatal sepsis could be due to differences in geographic region, level of technological advancement available for neonatal sepsis management, and adherence level to infection prevention and control practices. The high prevalence of *S. aureus* underscores its current reemergence as a leading pathogen in neonatal sepsis, highlighting its persistent role in neonatal mortality and morbidity.

The present study observed high resistance of *S. aureus* isolates to erythromycin (87.0%), cefoxitin (83.0%), ampicillin (64.0%) and gentamicin (64.0%). Similar high resistance rates to ampicillin and cephalosporins were reported in a study conducted in the southern part of Nigeria (4). In a study conducted in

Ethiopia, high resistance rate among *S. aureus* isolates was also reported with the highest resistance to ciprofloxacin (19). In addition, a systematic analysis of *S. aureus* resistance in Nigeria reported high resistance rates to ampicillin (68,0%) and third-generation cephalosporins (69.0%) (5). These findings indicate an increasing trend of multidrug resistance among clinical isolates and may reflect widespread empirical antibiotic use and limited antimicrobial stewardship practices.

This study found the prevalence of methicillin resistance among *S. aureus* isolates to be 83.9%. This is higher than the study from Calabar, Nigeria, which reported a prevalence rate of 53.1% (6) and also significantly higher than 12% prevalence rate reported in a study conducted in Uganda (20). A study conducted in Zaria reported MRSA prevalence to be 36% (8). The high rate of MRSA among *S. aureus* population in our study could be the leading factor for therapeutic difficulty and longer hospital stay in the SCBU of the facility.

The high prevalence of MDR (74.2%) observed in our study is higher than that of the study on neonatal infection conducted in northeastern Nigeria, where MDR *S. aureus* strains accounted for 36.0% of isolates (8), but less than the rate in the study from Ethiopia (21), where MDR rate was more than 90%. A study from China (22) reported MDR rate in *S. aureus* isolates to be higher than 70%, which agrees with the rate reported in our study. This high rate complicates empirical therapy and has been reported to be associated with prolonged hospitalization and increased mortality in neonatal intensive care settings.

The MAR index values obtained in this study were predominantly higher than 0.2, indicating that the isolates originated from environment with frequent antibiotic exposure. A study evaluating MAR indices of *S. aureus* isolates from a tertiary healthcare facility in Nigeria reported MAR indices of the isolates to be higher than 0.2 (6). Another study reported that about 90.8% of *S. aureus* isolates had MAR index higher than 0.2 (3), suggesting sustained antibiotic pressure in clinical environments. In yet another study from Lagos State, Nigeria, MAR index above 0.2 was reported in 77.5% of *S. aureus* isolates (23). A study in Uganda (20) reported 41.0% of *S. aureus* isolates to have MAR index of 0.2. The high MAR index and MDR observed in our study and in other studies may have occurred from frequent empirical antibiotic administration that created 'selection pressure' associated with prolonged hospitalization in neonatal units.

The lack of association between MDR and MRSA in this study does not undermine the therapeutic significance of MRSA or other resistant bacteria in newborn infections. Methicillin-resistant *S. aureus* continues to be a significant pathogen in neonatal intensive care units, associated with high mortality, morbidity, prolonged hospitalizations, and increased healthcare costs (8). Consequently, ongoing surveillance of resistance patterns is crucial for efficient antimicrobial stewardship and prevention of nosocomial infections.

One limitation of this study is the absence of molecular methods for confirmation of MRSA as these isolates were identified using phenotypic techniques, while the gold standard method for identifying MRSA, polymerase chain reaction (PCR) detection of *mecA* gene, was not available. The epidemiology and transmission dynamics of *S. aureus* associated with newborn sepsis would be better understood by future research using molecular methods, such as *mecA* gene identification and genotypic characterization of other resistance determinants.

Conclusion:

Nevertheless, the findings of this study confirm that *S. aureus* was the most common causative bacterial agent of neonatal sepsis in the study area. The study also found high level of phenotypic antibiotic resistance in *S. aureus*, particularly to the most commonly used antibiotics. These results underscore the need for strengthened antimicrobial stewardship programs, routine AMR surveillance, and enhanced infection prevention strategies in neonatal care settings.

Contributions of authors:

HAB conceived and designed the study, collected data, performed laboratory analysis, analyzed the data and drafted the manuscript; BA provided overall supervision of research activities, provided scientific guidance and approved manuscript submission; HMR provided guidance and revised manuscript for scientific accuracy; AS supervised data analysis and interpretation of results and reviewed manuscript for important intellectual contents. All the authors read and approved the manuscript submitted for publication.

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Conflict of interest

No conflict of interest is declared

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Short Communication **Open Access**

Dual *vanA*/*vanB* gene-carrying vancomycin-resistant *Staphylococcus aureus*: An emerging threat from northeast Nigeria

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Abstract:

Background: Vancomycin is an antibiotic of last resort for the treatment of multidrug resistant *Staphylococcus aureus* including methicillin resistant *S. aureus* (MRSA). The emergence of vancomycin resistant *S. aureus* (VRSA) is a major threat to global healthcare with devastating implications. In Nigeria, data on VRSA is scarce while the molecular characterization of the organism from Northeast region is nearly absent. This current is aim to detect vancomycin resistance among MRSA isolates from Gombe State, Nigeria, and identify the genetic determinants involved.
Methodology: This was a laboratory-based study of 70 confirmed *S. aureus* isolates which were screen for methicillin resistance using the modified Kirby-Bauer disc diffusion method. Vancomycin resistance screening was conducted using the E-Test Strip and micro broth dilution method according to CLSI guideline (MIC ≥16 µg/mL). Multiplex PCR was used to detect the presence of *vanA* and *vanB* genes in isolates that were phenotypically resistant to vancomycin.
Results: Out of the 70 isolates, 15 (21.4%) were methicillin resistant. Vancomycin resistance was observed in 5 (33.3%) and 3 (20.0%) of the MRSA isolates using E-test strip and micro broth dilution methods respectively. Molecular detection of vancomycin resistance gene(s) among the 5 VRSA isolates revealed 2 (40.0%) had the unusual dual carriage of *van A* and *van B* genes. No vancomycin intermediate resistance was detected.
Conclusion: This study is among the earliest report from the northeastern part of Nigeria that investigated VRSA and to the best of our knowledge the first documented evidence of dual carriage of *vanA* and *vanB* in VRSA in the region. Thus; these findings highlight the need for urgent antimicrobial surveillance and effective infection control measures to tackle the dissemination of the highly resistant *S. aureus* strains.

Keywords: *Staphylococcus aureus*; MRSA; VRSA; Antimicrobial resistance

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Staphylococcus aureus résistant à la vancomycine porteur des gènes *vanA* et *vanB*: une menace émergente dans le nord-est du Nigéria

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Résumé:

Contexte: La vancomycine est un antibiotique de dernier recours pour le traitement des infections à *Staphylococcus aureus* multirésistant, notamment les souches résistantes à la méthicilline (SARM). L'émergence de *S. aureus* résistant à la vancomycine (VRSA) constitue une menace majeure pour la santé publique mondiale, avec des conséquences dévastatrices. Au Nigéria, les données sur le VRSA sont rares et la caractérisation moléculaire de cet organisme dans la région du Nord-Est est quasi inexistante. Cette étude vise à détecter la résistance à la vancomycine parmi les isolats de SARM de l'État de Gombe, au Nigéria, et à identifier les déterminants génétiques impliqués.

Méthodologie: Il s'agit d'une étude en laboratoire portant sur 70 isolats de *S. aureus* confirmés, testés pour leur résistance à la méthicilline par la méthode de diffusion sur disque de Kirby-Bauer modifiée. Le dépistage de la résistance à la vancomycine a été réalisé à l'aide de la bandelette E-Test et de la méthode de microdilution en bouillon, conformément aux directives du CLSI (CMI≥16µg/mL). Une PCR multiplex a été utilisée pour détecter la présence des gènes vanA et vanB dans les isolats phénotypiquement résistants à la vancomycine.
Résultats: Sur les 70 isolats, 15 (21,4%) étaient résistants à la méthicilline. Une résistance à la vancomycine a été observée chez 5 (33,3%) et 3 (20,0%) des isolats de SARM, respectivement par la méthode E-test et la méthode de microdilution en bouillon. La détection moléculaire du ou des gènes de résistance à la vancomycine parmi les 5 isolats de VRSA a révélé que 2 (40,0%) présentaient le portage inhabituel des gènes *vanA* et *vanB*. Aucune résistance intermédiaire à la vancomycine n'a été détectée.
Conclusion: Cette étude est parmi les premières menées dans le nord-est du Nigéria sur les VRSA et, à notre connaissance, constitue la première preuve documentée du portage simultané des gènes vanA et vanB chez les VRSA dans la région. Ces résultats soulignent donc la nécessité d'une surveillance antimicrobienne urgente et de mesures efficaces de contrôle des infections pour lutter contre la dissémination des souches de *S. aureus* hautement résistantes.

Mots-clés: *Staphylococcus aureus*; SARM; VRSA; résistance aux antimicrobiens

Introduction:

Staphylococcus aureus is a human pathogen capable of causing variety of infections from mild skin infections to life threatening sepsis, endocarditis and pneumonia (1). Its clinical relevance increases due to its ability to resist multiple antibiotics (2). The global proliferation of methicillin-resistant *S. aureus* (MRSA) since the 1960s has profound concerns due to increased morbidity, mortality, and healthcare costs (2).

For decades, vancomycin has serves as the potent antibiotic for the treatment of MRSA infections often as a last resort antibiotic (3). However, the global emergence of strains resistant to vancomycin which was first documented in the United States in 2002 has raised substantial alarm (4). Since then, VRSA has been reported in many countries including India, Iran, and South Africa, often in patients with prolonged hospital stay and/or prior vancomycin exposure (4,5). VRSA though, still comparably rare than vancomycin-intermediate *S. aureus* (VISA), signifies a shrinking therapeutic option, leaving clinicians with limited and/or costly less accessible alternatives such as linezolid, daptomycin and others (6,7).

In Nigeria, the occurrence of VRSA is under reported, moreover the few available studies from south west and north central reported varying prevalence from very few to striking 29–36%, and often relying on single phenotypic identification without molecular confirmation (8, 9). Crucially, there is virtually no any rich molecular data from northeast Nigeria, where the prevalence patterns may vary significantly. A recent study in the region detected *vanA* alone but did not detect the presence of *vanB* leaving a gap in the molecular confirmation of the organism (8). In contrast, our study employed both E-test and broth dilution for phenotypic identification and multiplex PCR for *vanA* and *vanB* detection.

Thus, this research provides an insight on molecular identification of VRSA, and to the best of our knowledge it is the first report from northeast region to document dual carriage of *vanA*/*vanB* in VRSA isolates. Our findings contribute with a data that will breach an essential gap in national antibiotics resistance surveillance thus, strengthening the antibiotic stewardship and infections control program.

Materials and method:

Study setting:

This study was conducted in Gombe State, northeastern Nigeria, using clinical isolates obtained from Federal Teaching Hospital Gombe. Previous phenotypic identification of *S. aureus* was carried out at the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria. The laboratory is equipped for routine bacteriological analyses.

Molecular confirmation of the presumptive *S. aureus* isolates was subsequently performed at Molecular Laboratory of Medical Microbiology Department, Gombe State University, using polymerase chain reaction (PCR) targeting the *S. aureus*-specific *nuc* gene. The laboratory is equipped with modern molecular biology facilities for DNA extraction, PCR amplification, and gel electrophoresis, ensuring accurate species confirmation before subsequent molecular analyses of antimicrobial resistance genes.

Study design:

This laboratory-based cross-sectional study was carried out where a total of 70 *S. aureus* isolates, previously identified and confirmed as stated above, were analyzed for vancomycin resistance.

Screening for methicillin resistant *Staphylococcus aureus* (MRSA):

To screen for methicillin resistance, antimicrobial susceptibility testing (AST) was carried out using the Kirby Bauer disc diffusion method on Mueller Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (10). Methicillin resistance was detected using cefoxitin (30 µg) discs.

Vancomycin susceptibility testing:

Vancomycin susceptibility of MRSA isolates was evaluated by two complementary methods; E-test strip and broth microdilution method. The minimum inhibitory concentrations (MICs) of the MRSA isolates for vancomycin were determined using vancomycin E-test strip (Oxoid, England). A 0.5 McFarland standard suspension of the isolates was made, and a lawn culture was done on Muller-Hinton agar plate. Test strips were placed on the inoculated agar plates and incubated at 37°C for 24 hours. The vancomycin MIC was read on the strip at the point where the ellipse of inhibition meets the strip, according to CLSI guideline (10).

For the broth microdilution method, the MIC of vancomycin for the MRSA isolates were also determined by the reference broth microdilution method using cation-adjusted Mueller Hinton broth, as recommended by CLSI (10). Serial two-fold dilutions of vancomycin (0.25–64 µg/mL) were prepared in 96-well microtitre plates, inoculated with 5×10^5 CFU/mL of each isolate, and incubated at 35°C for 24 h. The MIC was defined as the lowest concentration of vancomycin that absolutely inhibited visible growth.

In accordance with the CLSI breakpoints, isolates with MIC ≤ 2 µg/mL were categorized as vancomycin-susceptible *S. aureus* (VSSA), those with MIC 4–8 µg/mL as vancomycin-intermediate *S. aureus* (VISA), while those with MIC ≥16 µg/mL as vancomycin-resistant *S. aureus* (VRSA).

Molecular detection of resistance genes:

Genomic DNA was extracted from the MRSA isolates that showed phenotypic resistance to vancomycin using the Qiagen DNA extraction kit (Qiagen, Germany). Multiplex PCR was performed to detect *vanA* (732 bp) and *vanB* (635 bp) genes using primers described in Table 1 (11). The PCR reaction mixture contained DreamTaq polymerase, specific primers, template DNA, and nuclease-free water. Amplification was performed in a thermal cycler with the following program: initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were separated by electrophoresis on 1% agarose gels stained with ethidium bromide and visualized under UV light.

Data analysis:

Data were analyzed using SPSS version 21.0 (IBM, USA). Frequencies and percentages were calculated for categorical variables, and level of agreement were assessed using Kappa coefficient tests. Statistical significance was set at $p<0.05$.

Results:

Prevalence of methicillin resistance among *S. aureus* isolates:

Out of the 70 confirmed *S. aureus* isolates, 15 (21.4%) were phenotypically identified as methicillin-resistant *S. aureus* (MRSA), while 55 isolates (78.6%) were identified as methicillin-susceptible *S. aureus* (MSSA) as shown in Fig 1.

Table 1: Primers used for amplification of *vanA* and *vanB* genes in *Staphylococcus aureus* isolates

S/N	Primer	Forword	Reverse	Size (kb)	Reference
1	vanA	GGGAAAACGACAATTGC	GTACAATGCGGCCGTTA	732	11
2	vanB	ATGGGAAGCCGATAGTC	GATTTCGTTCCTCGACC	635	

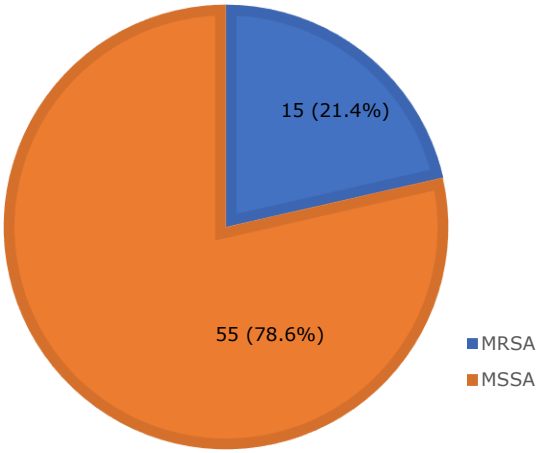


Fig 1: Prevalence of methicillin resistance among *Staphylococcus aureus* isolates

Results of vancomycin susceptibility testing:

Vancomycin susceptibility testing of the 15 MRSA isolates showed variable results depending on the method employed (Table 2). Using the E-test method, 5 (33.3%) isolates showed resistance to vancomycin, with MIC values ranging from 16 to 64 µg/mL. Furthermore, broth microdilution confirmed vancomycin resistance in only 3 (20.0%) isolates, while 12 (80.0%) were susceptible to vancomycin, All the 3-broth microdilution-confirmed VRSA isolates were also detected by the E-test. No vancomycin-intermediate *S. aureus* (VISA) isolates were detected.

Comparison of the two methods for vancomycin resistance detection demonstrated an observed agreement of 86.7% (13/15 isolates). Cohen's Kappa coefficient was 0.667, indicating substantial agreement between the methods.

Detection of vancomycin resistant genes:

Among the five phenotypically identified VRSA isolates, multiplex PCR detected concurrent carriage of the *vanA* and *vanB* genes in two isolates (40.0%). However, no *vanA* or *vanB* genes were detected in the remaining three isolates (60.0%), suggesting that alternative mechanisms of vancomycin resistance may be responsible for the observed phenotypic resistance (Table 3 and Fig 2).

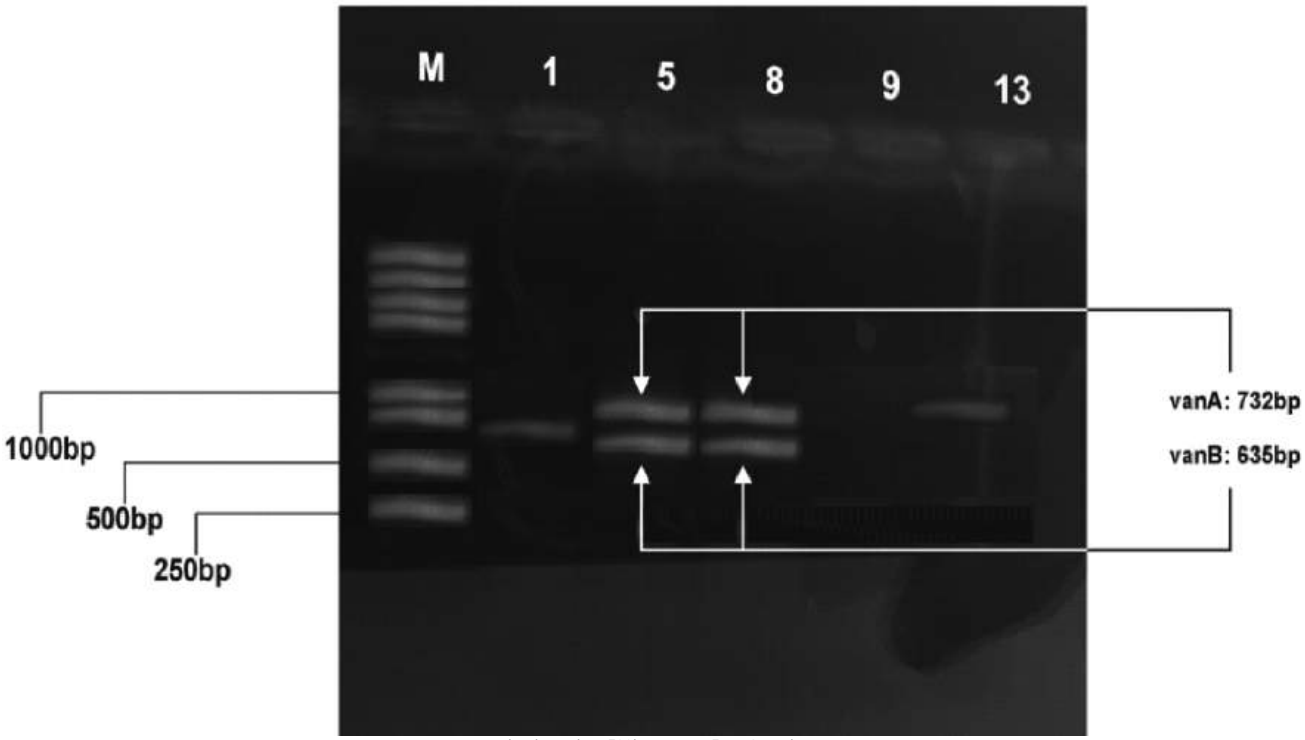
Table 2: Comparison of vancomycin resistance detection by E-test and broth microdilution

Method	Number of *S. aureus* isolates tested	Number of VRSA (%)	Number of VSSA (%)	Number of VISA (%)
E-test	15	5 (33.3)	10 (66.7)	0 (0.0)
Broth microdilution	15	3 (20.0)	12 (80.0)	0 (0.0)

VSSA=Vancomycin sensitive *Staphylococcus aureus*; VISA= Vancomycin intermediate *Staphylococcus aureus* VRSA= VISA= Vancomycin resistant *Staphylococcus aureus*; κ = 0.667 (kappa coefficient)

Table 3: Carriage of vancomycin resistance genes among phenotypic VRSA isolates

Van gene detected	Number of VRSA isolates tested	Number positive for gene (%)
vanA	5	2 (40.0)
vanB	5	2 (40.0)
vanA + *vanB* dual carriage	5	2 (40.0)



Lane M: Molecular Lader 1500bp; Lane 1-5: VRSA isolates

Fig 2: Agarose gel electrophoresis showing multiplex PCR amplification of *vanA* (732 bp) and *vanB* (635 bp) genes in VRSA isolates

Discussion:

This laboratory-based study determined vancomycin resistance among MRSA isolates from Gombe, northeast Nigeria. In the study, MRSA accounted for 21.4% of the 70 confirmed *S. aureus* isolates. Among these, vancomycin resistance was detected in 33.3% and 20.0% of the isolates using the E-test and broth microdilution methods, respectively. Molecular analysis further revealed the dual carriage of *vanA* and *vanB* genes in 40.0% (2 of 5) of the VRSA isolates**.** To the best of our knowledge, this is the first documented reports of such dual carriage in this region.

The detection of VRSA in this study adds to the limited body of data available in Nigeria. Previous studies have shown variable prevalence rates which depend on the study location and methodology employed in each study. For instance, Olufunmiso et al., (12) reported the prevalence of vancomycin resistance among *S. aureus* isolates in Southwestern Nigeria using agar dilution method, as 44.5%. while, Bamigboye et al., (13) reported a prevalence of 11.5% and 1.4% for VISA and VRSA respectively in a study conducted using E-test in Osogbo, southwest Nigeria but none of the isolates harbored either *vanA* or *vanB* genes. In contrast, Adamu et al., (8) reported an incidence rate of 45.5% of VRSA phenotypically by macrobroth dilution, of which *vanA* was detected in only 20% of the isolates although the study did not investigate the presence of *vanB*. Our findings demonstrate both phenotypic confirmation (using two independent methods) and molecular characterization, highlighting a more comprehensive approach. Furthermore, the detection of *vanB*, in addition to *vanA*, represents rare dual carriage of the genes in a single isolate.

Globally, the distribution of VRSA based on meta-analysis data showed North America, Europe and Asia with the prevalence rate of 3.6%, 1.8% and 1.2% respectively which remain rare but highly significant (4). However, higher rates have occasionally been reported from studies conducted in Africa, where antibiotic misuse and overuse is reported to be high (14-17). The finding from of this study is considerably higher than most international reports and aligns with earlier suggestions that some countries in Africa carry the highest burdens of AMR worldwide (18).

In addition, the detection of dual *vanA* and *vanB* genes carriage in two isolates in this study represent an important finding. Dual gene carriage is uncommon even in global literature, where most VRSA cases are attributed solely to *vanA* acquisition from *Enterococcus* species (19,

20). The coexistence of *vanA* and *vanB* in the same isolate may suggests complex genetic exchange events and may enhance bacterial survival under vancomycin pressure (20,21). To our knowledge, as stated earlier this is the first documented evidence of dual *vanA/vanB* VRSA isolates in northeast Nigeria, underscoring a worrying trend that requires close monitoring. The presence of VRSA in this setting is alarming, given that vancomycin remains a critical last-resort drug for MRSA infections (22,23).

The emergence of isolates carrying dual resistance genes further threatens therapeutic efficacy and highlights the risk of treatment failure which may leads to high rate of morbidity and mortality. These findings reiterate the need for strengthening of antimicrobial stewardship programs, routine molecular surveillance of resistance genes, and reinforcement of infection control practices in Nigerian healthcare facilities. Policymakers should prioritize investment in diagnostic capacity, antibiotic regulation, and public health awareness to curb the spread of these highly resistant strains.

Conclusion:

This study provides one of the earliest molecular reports of vancomycin-resistant *Staphylococcus aureus* (VRSA) from northeast Nigeria. Among MRSA isolates, 20.0% were confirmed as VRSA by broth microdilution, while molecular screening revealed dual carriage of *vanA* and *vanB* genes in a subset of the isolates. The coexistence of both resistance determinants is rare and raises serious concern for treatment outcomes and infection control.

Our findings underscore urgent need for continuous molecular surveillance of *S. aureus* resistance, robust antimicrobial stewardship programs, and strict infection prevention measures in healthcare facilities across Nigeria. Without decisive action, the spread of such highly resistant strains could severely limit therapeutic options and escalate the public health burden.

Contributions of authors:

HJA conceptualized the study and conducted all laboratory investigations. HJA and AH performed the data analysis and prepared the manuscript. LG, YAA, and IHI supervised the study and critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

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Letter to the Editor **Open Access**

Molecular detection is not diagnosis: A caution for antifungal stewardship as PCR testing expands in African clinical microbiology laboratories

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La détection moléculaire ne constitue pas un diagnostic: une mise en garde concernant le bon usage des antifongiques face à l'expansion des tests PCR dans les laboratoires de microbiologie clinique africains

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Dear Editor,

Molecular diagnostics for vulvovaginal candidiasis (VVC), real-time PCR assays in particular, are becoming more accessible across African clinical microbiology laboratories, offering same-day species-level detection at sensitivities that substantially exceed conventional microscopy (1,2). This is, on balance, a welcome development for a region where non-*albicans Candida* (NAC) species and antifungal resistance are disproportionately prevalent and where species identification has historically depended on culture alone (3,4). We write to flag a specific risk this transition carries if adopted without an accompanying interpretive safeguard.

Molecular assays detect *Candida* nucleic acid. They do not, by themselves, distinguish active symptomatic infection from asymptomatic colonization (5). This distinction is not academic. A substantial proportion of women harbour *Candida* as a vaginal commensal without symptoms at any given time, and colonization prevalence in some African cohorts has been reported to be considerable (6). As molecular testing becomes more available and more sensitive than the culture- and microscopy-based methods it is replacing, the population of women receiving a positive result will necessarily include more asymptomatic carriers than before, not because colonization is increasing, but because detection is improving.

The clinical consequence, if this is not managed deliberately, is straightforward: a positive PCR result in an asymptomatic woman, treated as if it were diagnostic of disease, produces unnecessary antifungal exposure (5). In a region already documented to have a high prevalence of intrinsically less azole-susceptible NAC species and, in at least one recent case series, direct evidence of antifungal-resistant *Candida albicans* in recurrent disease, this is

precisely the wrong direction of travel (3,4). Repeated, poorly indicated antifungal exposure is itself a resistance-selecting pressure, and the populations least able to absorb a rising resistance burden, owing to limited access to newer, non-azole systemic agents, are the ones this diagnostic transition is meant to serve (7).

We are not arguing against molecular diagnostics; the sensitivity gains are real and clinically valuable, particularly for detecting mixed infections and NAC species that culture-based algorithms in resource-limited settings may miss (1,2). We are arguing that the interpretive framework has to travel with the technology. A positive molecular result should prompt clinical correlation, not automatic treatment, and laboratories introducing PCR-based VVC testing should report results in a way that makes this explicit to the requesting clinician rather than issuing a bare "detected/not detected" result that invites reflexive prescribing.

Antifungal stewardship frameworks specific to VVC remain underdeveloped generally, and essentially undeveloped in African settings specifically (7,8). As molecular diagnostics expand across the continent, we suggest this is the moment to build the interpretive safeguard in from the start, rather than retrofitting it after a period of avoidable antifungal overuse. Concretely, we recommend that laboratories adopting PCR-based VVC testing pair every result with a standardized note stating that detection alone does not confirm disease, and that professional bodies coordinating this rollout consider issuing regional guidance on result interpretation and clinician communication before, rather than after, adoption becomes widespread.

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