ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

JUL 2026; VOLUME 27; NUMBER 3; PAGES 247-407

Official Publication of the African Society for Clinical Microbiology

ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (AJCEM)

https://www.ajol.info/index.php/ajcem and https://www.afrjcem.org

Editorial Board

All correspondence to Editor-in-Chief
Department of Medical Microbiology,
LAUTECH Teaching Hospital,
PMB 4007, Ogbomoso, Nigeria
Email: afrjcem@gmail.com and ajcem2019@gmail.com
Mobile: +234 (0) 8033436344
OR
Department of Medical Microbiology,
College of Health Sciences,
LAUTECH, PMB 4000, Ogbomoso, Nigeria
E-mail: sstaiwo@lautech.edu.ng

ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (AJCEM)

https://www.ajol.info/index.php/ajcem and https://www.afrjcem.org

July 2026; Volume 27: Number 3: Pages 247-407

TABLE OF CONTENTS

REVIEW ARTICLES

ORIGINAL ARTICLES:

SHORT COMMUNICATIONS:

ISSN 1595-689X

ERRATUM

CORRIGENDUM

Ayeni et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 247 - 262 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2482. https://www.ajol.info/index.php/ajcem

Review Article **Open Access**

Malaria: A narrative review of historical efforts in prevention and control

*[1,2]Ayeni, A. E., [3]Azuh, V. O., [1]Oluwatoba, O. A., and [1,4]Fayemiwo, S. A.

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Medicine, University of Ibadan, Nigeria
[2]Department of Epidemiology and Medical Statistics, Faculty of Public Health, College of Medicine, University of Ibadan, Nigeria
[3]Biorepository and Clinical Virology Laboratory, College of Medicine, University of Ibadan, Nigeria
[4]Clinical Microbiology Unit, Infectious Disease Institute (IDI), College of Medicine, University of Ibadan, Nigeria
*Correspondence to: ayenieugene@gmail.com

Abstract:

Malaria is an ancient disease that continues to exert a significant toll on the health of many populations worldwide. Since historic times, when its seasonality, recurrent fever and chills, were documented, various methods for its control have been implemented based on then-extant scientific knowledge, technological advancement and the understanding of its pathogenesis and epidemiology. As knowledge of the disease increased, approaches for its control were modified or improved to reduce disease burden and eliminate the disease. Today, our current knowledge of malaria has afforded newer approaches for prevention and control, including newer drugs and procedures to monitor emerging threats and hence more effective control mechanisms. In this narrative review, we consider a brief history of malaria, with insights into malaria pathogenesis and an evolution of prevention and control measures into the present day.

Keywords: Malaria prevention, malaria history, *Plasmodium*

Received Jan 8, 2026; Revised Feb 28, 2026; Accepted Feb 29, 2026

Paludisme: Revue narrative des efforts historiques de prévention et de contrôle

*[1,2]Ayeni, A. E., [3]Azuh, V. O., [1]Oluwatoba, O. A., et [1,4]Fayemiwo, S. A.

[1]Département de Microbiologie Médicale et de Parasitologie, Faculté des Sciences Cliniques Fondamentales, Collège de Médecine, Université d'Ibadan, Nigéria
[2]Département d'Epidémiologie et de Statistiques Médicales, Faculté de Santé Publique, Collège de Médecine, Université d'Ibadan, Nigéria
[3]Biorépositoire et Laboratoire de Virologie Clinique, Collège de Médecine, Université d'Ibadan, Nigéria
[4]Unité de Microbiologie Clinique, Institut des Maladies Infectieuses (IDI), Collège de Médecine, Université d'Ibadan, Nigéria
*Correspondance à: ayenieugene@gmail.com

Résumé:

Le paludisme est une maladie ancienne qui continue d'avoir un impact considérable sur la santé de nombreuses populations à travers le monde. Depuis l'Antiquité, date à laquelle sa saisonnalité, ses épisodes de fièvre récurrente et ses frissons ont été documentés, diverses méthodes de lutte contre le paludisme ont été mises en œuvre, s'appuyant sur les connaissances scientifiques de l'époque, les progrès technologiques et la compréhension de sa pathogénie et de son épidémiologie. À mesure que la connaissance de la maladie s'est approfondie, les approches

de lutte ont été modifiées et améliorées afin de réduire la charge de morbidité et d'éradiquer la maladie. Aujourd'hui, nos connaissances actuelles sur le paludisme ont permis de développer de nouvelles approches de prévention et de lutte, notamment de nouveaux médicaments et des procédures de surveillance des menaces émergentes, et donc des mécanismes de lutte plus efficaces. Dans cette revue narrative, nous présentons un bref historique du paludisme, en apportant un éclairage sur sa pathogénie et sur l'évolution des mesures de prévention et de lutte jusqu'à nos jours.

Mots-clés: Prévention du paludisme, historique du paludisme, *Plasmodium*

Introduction: A brief history of malaria

Malaria is an ancient disease with an early history as far back as 500 BC. The first reference to the disease mentioned its characteristic recurrent fever and chills, and was documented in the early Chaldean, Chinese, and Hindu writings (1). Ancient philosophers such as Hippocrates and Celsus alluded to malaria in their writings (2,3). Others mentioned its seasonality, the disease being more frequent during the late summer and early autumn (1). In the sacred Vedas of the Indians, it was referred to as the "king of diseases" and was associated with spleen enlargement, and its symptoms were attributed to supernatural forces (4,5). Malaria holds an important place in the annals of history, being infamous as the infectious disease that has had the most devastating impact on man. It is believed to have contributed to the fall and conquest of nations, as soldiers fell and were subdued, not just by the enemy's bullet, but by the scourge of the disease (6). As Brabin (7) noted, it attacked all combatants during World War I, causing epidemics and severe consequences for troops and civilians. In the Desert Mounted Corps, a British Army group composed of three divisions of military men, about 19,652 of 40,000 soldiers were ejected due to *Plasmodium falciparum* malaria (8). In addition, immigrant troops from Africa and India brought in a large pool of malaria parasites, serving as a mobile reservoir that further worsened the landscape of the malaria scourge during the war (7).

Plasmodium ovale and *P. vivax*, capable of causing malaria relapse, played important roles in the war scenes by triggering persistent difficult-to-treat infections, hence causing the evacuation of a large number of sick troops from combat zones (8). As soldiers in malaria-plagued war zones wore out, they were replaced by non-immune troops, with far-reaching consequences. Furthermore, malaria epidemics aboard voyage ships were of note during the war (9). This play-out of events affected military planning and war strategies, consequently factoring into war outcomes. Sometimes, military operations provided the conditions that fostered the advancement of the disease. For example, soldiers were often disposed to construct underground channels, an environment conducive to water-logging, and that provided the ambience required for mosquito breeding (8). Towards the end of the war in 1918, the concurrent outbreak of influenza and malaria resulted in over 1000 deaths, with a disease-to-combat casualty ratio of about 37 to 1 (7). The impact of malaria was also seen in World War II, Korean and Vietnam wars where malaria drug scarcity was a bottleneck.

Due to frequent military personnel encounters with the scourge of malaria (and infectious diseases in general), they are regularly involved in infectious disease research, as these diseases often play a role in determining the out-come of wars. In 1880, Charles Louis Alphonse Laveran, a French Army surgeon, discovered the parasite, as he examined the blood of a febrile soldier in Algeria (4). He described the parasite as transparent, often crescent-shaped, or ovoid, and having darkly stained pigment granules towards the center (10). He named this parasite *Oscillaria malariae* (11), now called *Plasmodium*. Further research led him to identify four distinct forms of the parasite, later recognized as the parasite in different developmental stages. His discovery was phenomenal in light of the pervasive knowledge of his day; firstly, that malaria was caused by foul smears, and then, the more scientific proposition of a so-called *Bacillus malariae* aetiology (11).

In 1897, seven years after Laveran's initial discovery, Sir Ronald Ross of Britain, another Army surgeon, discovered the parasite in the intestine of mosquitoes and subsequently demonstrated its lifecycle through a series of experiments involving avian-infecting species of *Plasmodium* (4). Ross received the Nobel Prize in medicine in 1902, while Laveran received it in 1907 for their discovery of the parasite and the vector that transmits it. Moreover, further details of the parasite lifecycle, particularly of the exoerythrocytic phase of infection, were elucidated by Shortt and Garnham in 1947, while the identification and characterization of the dormant hypnozoite form was made by Krotoski in 1982 (11–13). Today, we now know that human malaria is caused by six species of *Plasmodium,* namely, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale curtisi*, *P. ovale wallikeri* and

P. knowlesi (14–16), and is transmitted by several species of the female *Anopheles* mosquito, notably by *Anopheles gambiae* and *Anopheles funestus* in West Africa.

Human malaria parasites:

Human malaria is caused by six species of *Plasmodium*. These parasites vary slightly in biochemical/molecular attributes, as well as the diseases they cause.

Plasmodium falciparum:

Plasmodium falciparum causes the most severe form of malaria. Following liver-stage infection, the parasites are released into the bloodstream, where they infect red blood cells (RBC) and produce high levels of blood-stage parasites (17). These parasites cause changes in the physiology of the red cells, modifying the red cell surface such that they stick to the wall of blood vessels, a phenomenon known as sequestration, and that contributes to disease pathology. Sequestration inhibits splenic clearance of the parasite, damages host cell endothelia and obstructs host microvasculature. These features are characteristic hallmarks of severe malaria caused by *P. falciparum*.

Plasmodium vivax:

Next to *P. falciparum*, *P. vivax* causes the highest number of malaria (18). It is the most widespread and significantly contributes to malaria morbidity, especially in Southeast Asia and South America, where it is prevalent. *Plasmodium vivax* has a dormant parasite stage, the hypnozoite, which can remain in the liver for months or years without causing symptomatic disease until it eventually re-enters the bloodstream to cause a relapse (19). *Plasmodium vivax* merozoites only infect reticulocytes, the juvenile form of the red blood cell. Furthermore, they require the Duffy antigen on the surface of red blood cells for invasion. This attribute is thought to contribute to a much lower prevalence of *P. vivax* in Africa, as majority of the population have no Duffy antigen on the red cell (20). *Plasmodium vivax* malaria is characterized by recurring fevers that occur every third day (approximately every 42-56 hours), called 'tertian fever'.

Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi:

Plasmodium ovale has traditionally been considered a single species and afterwards as members of the *ovale* subspecies; however, recent evidence has shown that these are two distinct species (21,22). Although both are identical in morphology and geographical distribution, they are genetically distinct (23,24). Both parasites are known to have the same course of infection: sporozoites can develop into dormant hypnozoites; they infect reticulocytes, and infected cells appear more prominent than usual (15,25). However, a study has highlighted slight differences in hypnozoite latency period in travelers' malaria (26), as well as higher thrombocytopenia in infection due to *Plasmodium ovale wallikeri* (27).

Plasmodium malariae:

Plasmodium malariae causes mild malaria symptoms, with recurrent fevers in a quartan manner, and tends to infect patients chronically (14). Chronic infection results from a slow parasite lifecycle (erythrocytic cycle every 72 hours) and result in lower parasitemia that the immune system can manage for long periods (15). Infection with *P. malariae* is associated with nephrotic syndrome with consequences for long-term kidney failure (28). Although, they have a limited geographical range than *P. falciparum* and *P. vivax*, *P. malariae* infection, transmission may persist undetected in unsuspecting geographical locations (28).

Plasmodium knowlesi:

Plasmodium knowlesi is a zoonotic malaria parasite that preferentially infects young red blood cells, but with time, can adapt to infect older cells (15). The disease presents with the classic symptoms of malaria, such as fever, chills and headache, and additional signs such as myalgia/arthralgia, nausea and vomiting, anorexia and jaundice (29). It is primarily a parasite of long-tailed and pig-tailed macaques, from which the parasite is transmitted to man; no human-human transmission has been recorded (15,16). Although, there is as yet no evidence of human-mosquito-human transmission of the parasite, studies have shown the biological possibility of this occurrence (30,31), and with increasing prevalence of Knowlesi malaria in parts of Asia, human-human transmission is feared to contribute to disease distribution (32).

Pathogenesis of malaria

The malaria lifecycle involves an alternation of parasite stages between the mosquito vector and humans (Fig 1). The different parasite developmental stages in humans involve the asexual sporozoite, merozoite, trophozoite, schizont and the gametocyte, while in the mosquito, the gametocyte, gametes, zygote, ookinete, oocyst and sporozoite are the different stages involved.

Pre-erythrocytic infection:

The lifecycle begins when an infected mosquito takes a human blood meal. As the infected mosquito takes the blood meal, it releases a hundred or more sporozoites into the blood stream (33). Blood feeding and sporozoite injection is facilitated by the mosquito saliva which contains several proteins, vasodilators, anticoagulants and immunomodulatory compounds, that prevent the otherwise formidable skin barrier from halting sporozoite entry and survival (34).

Our scientific understanding of the fate of injected sporozoites has improved greatly in the past few years, largely through studies on murine models of malaria. Approximately 50 % of injected sporozoites are arrested at the skin site of inoculation, where they exhibit a classical gliding locomotion with average speed decreasing over time (35). Of the remaining sporozoites, others are transported via the lymph to the skin-draining lymph nodes, where partial differentiation into parasite exoerythrocytic stage occur (35), while others penetrate blood vessel endothelia and are transported to the liver (36).

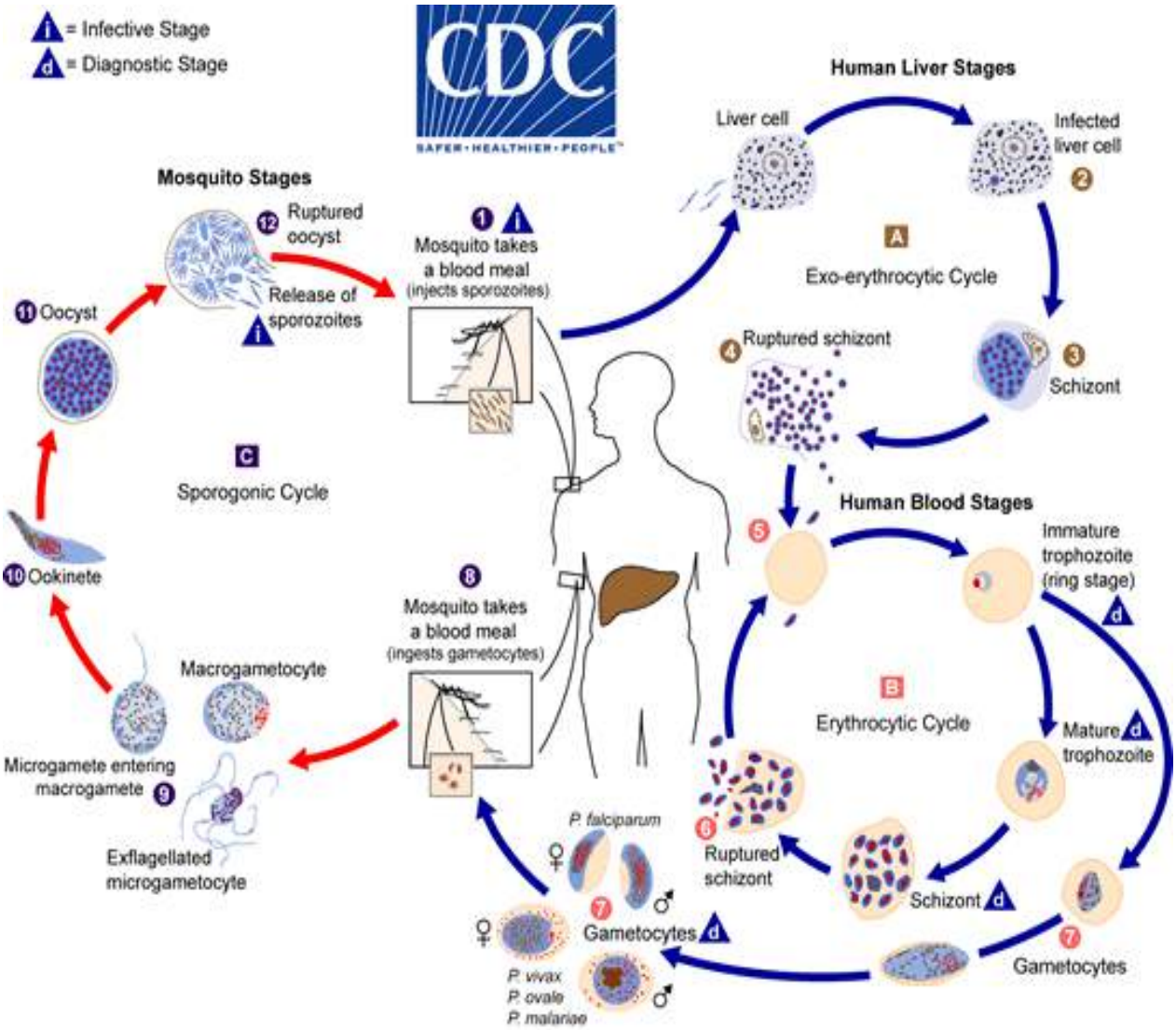


Fig 1: The Malaria Lifecycle. (Source: Center for Disease Control and Prevention)

In a recent study employing humanized mouse model of infection, *P. falciparum* sporozoite motility and penetration of blood vessels appear similar to those of the rodent parasites, *P. berhei* and *P. yoelii* (37). The study highlighted some interesting features of similarity at the initial phase of infection between the rodent- and human-infecting species of *Plasmodium*. In addition, parasite motility decreases upon close proximity and contact with blood vessels, a move thought to improve their interaction and possibly their penetration of blood vessels (38) for subsequent dissemination to the liver predilection site.

The migration from the loci of sporozoite inoculation to the liver is faced with several hurdles. First, sporozoites have to deal with the influx of immune cells, polymorphonuclear leukocytes, lymphocytes and monocytes due to mast cell degranulation (39). Phagocytic immune cells such as the macrophages, neutrophils and dendritic cells also play roles in interfering with sporozoites' migration. Likewise, immunoglobulins specific to the circumsporozoite protein (CSP) neutralize free sporozoites and impede their migration (40). Howbeit, the stealth mechanisms in *Plasmodium* prevent absolute neutralization by the effector activities of the immune system (36).

Moreover, as free sporozoites migrate to the liver, they bypass the immunologic activity of Kupffer cells using a mechanism known as *cell traversal*. Simply, the sporozoites enter a Kupffer cell at one end and exits at the other end, altering the plasma membrane integrity and destroying the cell in the process (41). As Kupffer cells line the sinusoidal covering of the liver, sporozoites have to do a lot of Kupffer cell traversing before eventually settling in a final host liver cell (34). In the hepatocyte, sporozoites are enclosed in a parasitophorous vacuole, where they multiply and are transformed into schizonts. For each infected hepatocyte, several thousands of merozoites are produced (15), and released as merosomes (41), a form of the parasite enclosed in host cell membrane. This enclosure in host cell membrane is thought to contribute to the ability of *Plasmodium* to evade the host immune system. As merosomes are released and escape the liver, they shed this enclosure and become free merozoites, ready to infect red blood cells.

Intraerythrocytic infection:

Invasion of erythrocytes by a merozoite is initiated by the merozoite surface proteins through direct interaction with the red blood cell (RBC) surface. The invasion is further facilitated by the merozoite's micronemes and rhoptries, organellar structures present in the merozoite, which also aid the formation of the parasitophorous vacuole where the parasite grows and multiplies (16). In addition, the parasite conditions the internal environment of the erythrocyte for its survival by exploiting RBC nutrients and exporting parasite-derived proteins that further drives the disease pathogenesis. RBC remodeling is anchored on parasite-induced protein targeting networks that sort and target proteins to specific subcellular locations in the host cell, including on the RBC membrane (42). This network includes *Maurer's clefts* and *J-dots,* membranous structures that are seen independent of the parasitophorous vacuole, and that contribute to protein transport to the RBC surface. In addition, the parasite degrades red cell haemoglobin within its digestive vacuole resulting in heme, a toxic by-product which is further metabolized into hemozoin (43). As RBC nutrients are utilized, the merozoite grows and develops into schizonts which are formed by multiple nuclear asexual divisions, thus producing 15-40 merozoites per infected red cell (16, 44).

This stage of the disease eventually produces the fevers, chills and rigors of clinical malaria as mature schizonts in multiple infected RBC synchronously rupture and release their constituent merozoites and pathogen-associated molecular patterns (PAMPs). The resulting sequelae often occur in varying degrees, dependent on the host immune response. The released merozoites are now ready to infect other red blood cells. Moreover, commitment to the production of sexual stage parasite occurs in some merozoites during blood stage infection. Simply, merozoites differentiate into gametocytes and are released as the schizont ruptures (45). This process in *P. falciparum* takes approximately 10-12 days during which the parasite undergoes different developmental stages in the spleen and bone marrow before the matured forms are released into blood circulation. These gametocytes circulate in the blood and are picked up by the mosquito during a blood meal.

Mosquito-stage infection:

Within the mosquito, gametocytes egress from infected red cells and develop into gametes. Exflagellation of male gametocytes results in eight microgametes whereas a female gametocyte develops to form one immotile macrogamete (45,46). The microgamete fertilizes the macrogamete, resulting in the production of a zygote, which then transforms into a motile ookinete. The ookinete penetrates the mosquito midgut wall by utilizing its lectin molecules and mosquito-derived sugars (40) and is afterward

transformed into an oocyst on the outer surface of the mosquito midgut. The oocyst generates thousands of sporozoites which penetrate the mosquito salivary gland, ready to be released into a human host during the mosquito's next blood meal.

Obstacles to malaria elimination as a public health problem:

The vaccine imbroglio:

The malaria parasite is complex, with a genome size of 23.3Megabases and over 5400 genes (47). This repertoire of genetic information (including several multigene families) informs the plethora of variant proteins/antigens expressed by the parasite during, and between each developmental stage. For example, the *var* family of genes encode up to 60 variants of a single protein, the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), in a single parasite, which contributes to antigenic variation and immune evasion (48).

This polymorphism in protein biology and attendant antigenic variation serves as a formidable obstacle to vaccine development. In addition, the variation in parasite gene expression between lifecycle stages contributes to the complexity. This complexity and dynamism in protein expression contributes to parasite complexity, phasic availability of antigens during infection, and provide stealthy cover from immune defense mechanisms (49). These complexities cause difficulty in mounting a sterilizing immunity against the parasite. The wide range of *Plasmodium* antigens exposed to the immune system, make it difficult to identify the best antigen vaccine candidates, and an immune system response directed at one stage of the parasite is rendered ineffective during another stage of the parasite (50).

Parasite drug resistance:

Plasmodium species resist antimalarials through changes to the genes encoding various transport proteins that transport various solute molecules within the parasite. Solute transport typically involves nutrients, metabolic by-products and xenobiotics, including substances that could potentially harm the parasite. Two key cellular compartments are target solute destination sites; the parasite digestive vacuole and the cytosol. The digestive vacuole is the primary site for haemoglobin digestion, and contains various hydrolytic enzymes of diverse classes that help accomplish hemoglobin digestion, heme detoxification and oxygen radical neuralization (43). Serving various purposes including the detoxification of harmful substances, the digestive vacuole is thus akin to the lysosome of other eukaryotic cells, where potentially toxic substances are shipped for processing (51).

PfMDR1 is an ATP-binding cassette transporter composed of 1,419 amino acids residues, with 12 putative transmembrane domains, present on the parasite's digestive vacuole (52). Its particular location on the lysosomal-type compartment of the digestive vacuole membrane makes it implicated in the transport of solutes, including antimalarial drugs (53). Although, the nature of single nucleotide polymorphisms (SNPs) in this gene will have differing effect on how antimalarials are transported, the general mechanism of action involves drug trafficking away from their target sites. This factor impacts on drug efficacy, offering the parasite resistance to the implicated drug.

There are five prevalent amino acid mutations in the *pfmdr1* gene, namely the N86Y and Y184F which according to epidemiological studies, are more prevalent in Asian and African parasites (52), and involves the substitution of asparagine for tyrosine in N86Y and tyrosine for phenylalanine in Y184F (51). The S1034C, N1042D and D1246Y mutations are more commonly found in South American parasites, and involve substitution of serine for cysteine, asparagine for aspartate, and aspartate for tyrosine at their respective codons (51). The N86Y and N1042D reduce parasite susceptibility to chloroquine and quinine respectively, and concomitantly increase susceptibility to lumefantrine and mefloquine, ACT partner drugs (53). Similarly, amodiaquine resistance is associated with 86Y, Y184 and 1246Y resistance alleles (54,55). Increased copy number of the gene is associated with mefloquine resistance and reduced lumefantrine susceptibility (56).

The PfCRT is composed of 424 amino acid residues arranged into 10 transmembrane domains. This transporter also sits on the parasite's digestive vacuole membrane, where it mediates drug trafficking from the parasite's intravacuolar compartment to the cytosol, potentially shuffling away drugs from their active site, for example shuffling chloroquine away from its heme target (57). Mutations in *Pfcrt* gene has been implicated in antimalarial drug resistance; the (*Pfcrt*-K76T) mutation, where lysine is substituted for threonine confers concomitant chloroquine and amodiaquine resistance (54,55); SVMNT allele for amodiaquine resistance; and *Pfcrt*-C101F for piperaquine resistance (58). The 7G8 isoform of the parasite contains five point mutations viz; C72S, K76T, A220S, N326D, and I356L, which are all revealed to line the central drug-binding cavity. Four of these mutations are required to confer chloroquine

resistance, although the K76T appears to be a key mutation (57), being conserved in chloroquine-resistant parasites, (59), and along with *pfmdr1* mutations, employed as a molecular marker in determining chloroquine resistance (60,61). Furthermore, levels of expression of the PfCRT determine chloroquine resistance, as low expression levels do not confer resistance to the drug.

PfCRT is opined to play roles in the trafficking of other metabolites; for example amino acids arising from haemoglobin breakdown, glutathione, iron, chloride and hydrogen ions, alkaloids, and acting as a non-specific cation channel (51,62). This transporter is therefore important to the normal physiological functioning of the parasite, being recently confirmed to play roles in the export of host-derived peptides of 4–11 residue length, with tendencies not congruent as a non-specific transporter of other metabolites and/or ions (63). In addition, other antimalarials such as primaquine, quinacrine, mefloquine, and low-weight peptides containing aromatic amino acids are able to competitively inhibit chloroquine from interaction with mutant PfCRT, indicating the role of this transporter in transporting additional antimalarial drugs and peptides outside the digestive vacuole (43).

Mutation in the *P. falciparum* Kelch 13 gene (*pfkelch13*) mediates resistance to artemisinin antimalarials, a discovery feared to sabotage the recent gains in malaria morbidity and mortality worldwide. *PfKelch13* has a single exon, and is located on chromosome 13 of *P. falciparum* (64). The gene encodes a protein of 726 amino acid residues, which is structurally configured into three protein domains: a sequence specific to *Plasmodium*; a BTB or POZ domain (a recognized signature for protein-protein interaction, also present in other proteins); and six repeats of the kelch motif, called the beta-propeller domain (65). Artemisinin resistance occurs due to mutations in this beta-propeller domain of kelch 13 (66). Over 100 of these mutations have been identified, with majority of the significant mutations occurring in Asia. C580Y, R539T, I543, and Y493H, are the most frequent of the mutations (67). Drug resistance (if not checked) will increase morbidity and mortality of the disease, serving as obstacles to malaria elimination/eradication.

Asymptomatic malaria reservoir pool:

Nigeria continues to bear a significant toll of the worldwide malaria burden. In 2021, Nigeria accounted for 27% of all malaria cases, the highest of any country in the world (68). Aju-Ameh (60) presents a unique perspective on asymptomatic malaria as a major challenge to malaria elimination. This author described a study in which 272 humans and 214 mosquitoes were investigated for the malaria parasite. This study revealed that humans had more parasite biomass (36.8%) compared to engorged anthropophagic female *Anopheles* mosquitoes (0.5%). This finding illustrates a higher prevalence of *Plasmodium*-infected humans than mosquitoes, and this is believed to be "the existing phenomenon in most scenarios" (69). These human parasite reservoirs are a potential source of continuous parasite transmission within the population.

As gametocytes are the parasite forms infective to mosquitoes, the presence of mature gametocyte in blood is crucial for a sustained parasite transmission (70). Moreover, establishing and quantifying gametocyte presence in blood is important to monitor transmission patterns and the potential infectiousness of asymptomatic individuals (71). Detection may be achieved by microscopy. However, very low gametocyte densities will require more sensitive approaches for their detection (72), especially in low transmission settings where gametocyte detection by microscopy is elusive. However, failure to detect gametocytes by microscopy or molecular methods does not exclude the possibility of malaria transmission, provided that other parasites forms are present. This is true because all malaria have the capacity to produce gametocytes (71). Evidently, in endemic settings, asymptomatic infections usually persist through the dry season, perpetuating disease transmission into the wet season (70,73, 74). Generally, the higher the circulating gametocyte numbers, the higher the infectiousness of an individual. And, although, higher gametocyte densities tends to higher infectiousness, submicroscopic gametocyte levels still efficiently transmits parasites to mosquitoes (74,75).

Deletions in *P. falciparum* Histidine-Rich Protein 2 and Protein 3 Genes:

The majority of rapid malaria testing for *P. falciparum* leverages the detection of histidine-rich protein 2 (68), a biomolecule produced in prolific amounts during asexual stage growth, with highest numbers occurring during the ring stage, and reduced accumulation during trophozoite and schizont stages of development (76). Its implementation in malaria rapid diagnostic testing has transformed the malaria diagnosis landscape, allowing for a more rapid diagnosis in community surveys and in malaria-endemic regions with a poor health-care infrastructure (77).

The occurrence of *P. falciparum* HRP-2 gene deletions was first observed in 2010 in

Peru, where samples positive for microscopy but negative by malaria rapid diagnostic test (mRDT) were scrutinized (78). In the study by Gamboa et al., in 2010 (79), 41% of retrospectively tested samples lacked the HRP-2 gene, and 21.6% of the samples lacked the HRP-3 gene, a closely related gene to the former, that produces a protein of similar structure and shared epitopes (79). The deletions have since then been detected in other regions such as in Ghana (80), Ethiopia (81), Nigeria (82), and in other regions of the world (83–85).

Parasites that do not express the HRP-2 gene or having HRP-2/3 gene deletions undermine the effectiveness of the HRP-2 based rapid testing, and interfere with prompt diagnosis and treatment, which are essential elements in the malaria response and elimination race. As HRP-3 can still be detected in HRP-based mRDT assays, this protein provides some usefulness in testing. However, concomitant deletion of both genes in parasite strains worsens case detection and paralyzes the usefulness of rapid testing employing the histidine-rich proteins.

Climate change and vector expansion:

Climate change and global warming is believed to be a driving factor in vector expansion to regions they are traditionally not present. For malaria, this is true for *Anopheles stephensi*, originally native to the Indian subcontinent, parts of the Middle East and south Asia regions, but now found in Africa. *Anopheles stephensi*, a malaria vector primarily found in parts of Southeast Asia, the Arabian Peninsula and the Middle East has recently arrived in Africa, reversing previous gains in selected regions and threatening to worsen the malaria crisis in new parts.

The first report of *A. stephensi* in Africa was in Djibouti in 2012, where the vector triggered an epidemic of *P. falciparum* (86). Before the vector's invasion of Djibouti, malaria was at the verge of elimination in the country, with only 27 cases reported in 2012. However, with the two epidemics of malaria in the country, over 3000 cases were recorded between 2013 and 2014; in the early 2013 epidemic, 1228 cases were reported, and in a second more severe epidemic spanning from late 2013 to early 2014, over 2017 cases were reported (86). Since then, malaria cases have increased in the region (Fig 2), with over 73000 cases reported in 2020 (87), as well as recorded yearly deaths (68). Since 2012 when *A. stephensi* entered Africa, it has spread to other countries, notably Ethiopia, Sudan and Somalia (88–90), and more recently Nigeria (91) and Kenya (92).

In Ethiopia, invasion of the vector has been linked to at least one outbreak of the disease (93), and reports of insecticide resistance (pyrethroids, organophosphates and carbamates) in all regions (68,94,95). Although it is generally unclear and somewhat variable what the overall role of *A. stephensi* is to malaria transmission in some of these regions, the Ethiopia epidemic, and the identification of circumsporozoite proteins in female *A. stephensi* mosquitoes through vector surveillance studies have indicated blood feeding on humans and some role in malaria transmission (86,88,95,96). Furthermore, it is feared that due to the unique invasive properties of this vector and the rapid urbanization in Africa cities, the recent gains in malaria control efforts could be sabotaged (97–99).

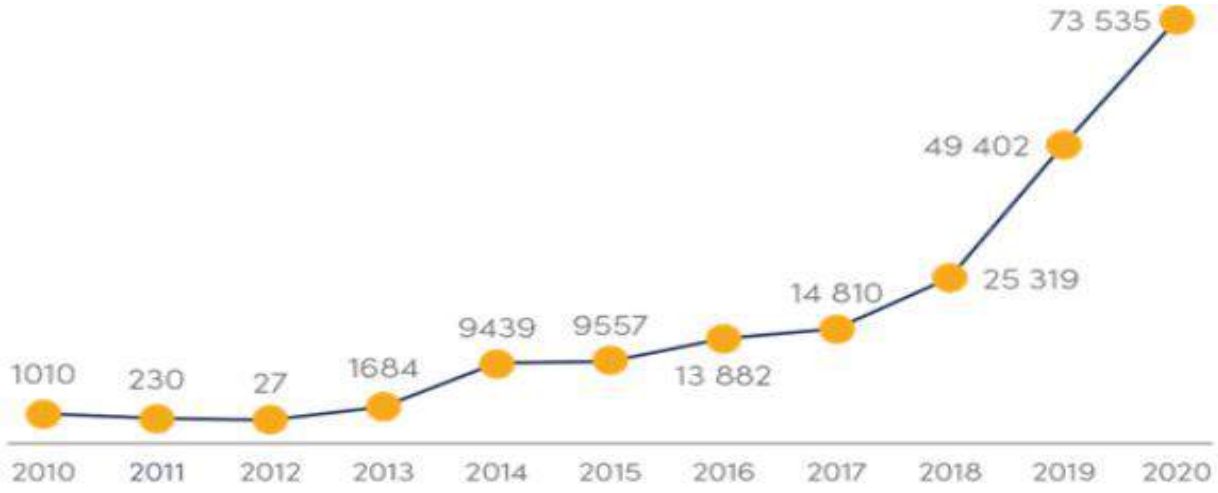


Fig 2: Impact of *Anopheles stephensi*'s invasion of Djibouti: From near elimination in 2012 to geometric increase in reported malaria through subsequent years [Source: WHO (116)]

Malaria control: Global targets and current efforts

Historical background of malaria control:

From the earliest times, malaria has been associated with stagnant water in swampy environments. In fact, the name "malaria" (*malaria*: Bad air) is the result of such association, when the Italians attributed the disease to the ill-smelling vapours from swamps (3). Although such attribution lacked precision, it reveals the appreciation of stagnant water as a factor in the cause of seasonal febrile illnesses, a distinctive feature of malaria. Ancient practices therefore included to a large degree, the control of malaria by draining/eliminating water from swamps, marshes, ponds or other body of stagnant water (2,100). This practice also occurred during warfare when soldiers fell to malaria (8), and still remains, as health education programmes often advice to clear and remove stagnant water around living premises, since these water enclosure serve as breeding sites for mosquito vectors.

As the etiology and lifecycle of the parasite were discovered in late 19th century, subsequent efforts in malaria control involved the use of larva-eliminating strategies and quinine administration in the early 20th Century, although quinine itself had been discovered earlier and used in the general treatment of intermittent fevers (4). The use of anti-larva strategies involved the larvicide Paris Green (copper acetoarsenite) and larvivorous fish (101).

In the 1950s, the WHO initiated the Global Malaria Elimination Programme (GMEP), which was intended to eradicate malaria globally (102). The initiative was based upon the discovery and the effectiveness of dichlorodiphenyltrichlorethane (DDT), an insecticide that found wide spread use during the World War II. However, the GMEP initiative failed to achieve its ultimate goal, although substantial malaria elimination was recorded in many countries. It came to an end in 1969, with the realization that sub-Saharan Africa, the seat of the malaria plague was not responsive to a large-scale eradication program such as GMEP (103). Moreover, due to a highly rigid approach, the use of a single elimination tool, and inadequate foresight and planning, the GMEP failed to deliver on its goal (102).

The next programme targeted towards malaria was the Roll Back Malaria (RBM) campaign, initiated in 1998 at the debut of Gro Brundtland's term as the Director-General of the World Health Organization (104). It was a joint effort of the WHO, United Nations International Children's Emergency Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank (105), born in the face of the high burden of malaria in sub-Sahara Africa, a region that then accounted for 90 % of the global malaria mortality. The initiative had a goal to reduce malaria morbidity and mortality by half in 2010 (106), through resource mobilzation and concerted partner efforts (103). The campaign failed for a number of reasons; leader ship, inadequate funding and drug resistance, amongst others (105,107–109).

Current goals/objectives of the WHO and associated World Organizations:

When the RBM campaign came to an end, 10 years after it began, the key UN agencies involved converged to discuss and implement a wider and more robust strategy for malaria elimination, the Global Malaria Action Plan (GMAP) (110). The GMAP was a recommendation from the RMB board, and was developed based on agreement between all participating bodies, and the collective input of international institutions and experts (111). Key intervention strategies included prophylactic treatment for pregnant women, use of ACT, use of insecticide treated nets, and indoor insecticide spraying (110).

In 2015 during the 68th World Health Assembly (Geneva), key stakeholders endorsed the Global Technical Strategy for Malaria 2016–2030 (GTS), the current strategy that outlines current milestones and goals for malaria control. These goals, with an overarching aim to reduce disease burden and eliminate malaria, are closely linked to specific goals of the United Nation's Sustainable Development Goals.

World Health Organization Global Technical Strategy (GTS) for Malaria:

The Global Technical Strategy (GTS) of the WHO (112) for malaria is anchored on five principles targeted at reducing disease burden and ultimately eliminating and eradicating the disease. The first strategy focuses on the use of combined intervention approaches that are adapted to the local context; the second is focused on leadership and community participation; the third is surveillance, monitoring and evaluation; the fourth is equity of access to health services, particularly for the most vulnerable; the fifth is innovation in intervention tools and approaches. These principles are intended to guide implementation of the strategy to achieve its vision, goals and milestones. The vision is a world free of malaria.

Table 1: Goals, Milestones and Targets of the World Health Organization Global Technical Strategy

Goals	**Milestones**		**Targets**
	2020	**2025**	**2030**
To reduce malaria mortality rates globally compared with 2015	At least 40%	At least 75%	At least 90%
To reduce malaria case incidence globally compared with 2015	At least 40%	At least 75%	At least 90%
To eliminate malaria in countries where it was transmitted in 2015	At least 10 countries	At least 25 countries	At least 35 countries
To prevent re-establishment of malaria in all countries that are malaria-free	Re-establishment prevented	Re-establishment prevented	Re-establishment prevented

The goals, milestones and targets are highlighted as follows; (1) To reduce malaria mortality rates globally compared with 2015. The specific milestones are to reduce malaria mortality by at least 40% by 2020, and by at least 75% by 2025. The 2030 target is to reduce the mortality by at least 90%; (2) To reduce malaria case incidence globally compared with 2015. The specific milestones are to reduce malaria case incidence by at least 40% by 2020, and by at least 75% by 2025. The 2030 target is to reduce mortality by at least 90%; (3) To eliminate malaria from countries in which malaria was transmitted in 2015. The specific milestones are to eliminate malaria in at least 10 countries by 2020, and at least 20 countries by 2025. The 2030 target is to eliminate the disease in at least 25 countries; and (4) To prevent re-establishment of malaria in all countries that are malaria-free. The specific milestones and 2030 target are to prevent re-establishment of malaria in all malaria-free countries. Within the context of the WHO's goals, elimination is defined as the interruption of the local transmission of a specified malaria parasite in a defined geographical area because of deliberate activities. The goals and targets of the GTS are summarized in Table 1.

United Nation's Sustainable Development Goals:

The Sustainable Development Goals are a shared scheme of the United Nations that is geared towards development and cuts across various strata, including education, environment and ecosystems, economic development and gender equality. Included in this multifaceted scheme is also the healthcare infrastructure (113). The SDGs are composed of 17 goals, 169 targets and 248 indicators; Goal 3, target 3.3 specifically addresses the scourge of malaria and other infectious diseases. It aims by 2030 to *"…end the epidemics of AIDS, tuberculosis, malaria, neglected tropical disease and combat hepatitis, water-borne diseases, and other communicable diseases."* (113).

Ending the malaria epidemic is also indirectly targeted by other goals of the SDGs; for example, goals 1, 2, 6, 9 address poverty, hunger, clean water and sanitation, and climate change respectively. These also address the scourge of malaria. Concerted efforts by partners and agencies will help achieve this feat.

Current efforts in achieving global targets:

Surveillance and monitoring:

Surveillance for vector distribution, drug efficacy and parasite resistance patterns are key approaches in driving the current global targets. As the malaria parasite and their vectors keep evolving, studies on their distribution and biological characteristics will keep the scientific and global malaria community abreast of the newest trends and effective strategies in intervention programs. For WHO, vector surveillance should entail the continued assessment of vector species present in particular geographical locations, their abundance and seasonality. For example, surveillance studies helped identify *Anopheles stephensi* in Africa and particularly in Nigeria (91). *Anopheles stephensi* is an efficient malaria vector traditionally native to regions of South Asia and the Arabian Peninsula, but now spreading in parts of Africa (99,114). In addition, relevant vector binomics such as host preference, place and time of bite, indoor and outdoor biting preferences, and susceptibility to currently used insecticides are monitored (112).

Regular monitoring such as this is necessary to keep abreast of the dynamics of vector distribution and their potential to transmit dis-

ease. In the face of environmental changes, such as global warming, and rapid urbanization, the landscape of malaria epidemiology is likewise changing, and hence the need for continuous monitoring. The likelihood of renewed malaria transmission in "malaria-free" regions is now feared as a subtle possibility. For example, the US, which has not recorded any indigenous malaria transmission in the last two decades recently experienced incidence of malaria (115), thus reemphasizing the need for a continuous monitoring even in regions that have eliminated the disease.

The WHO in 2022 launched a strategic plan to address the menace of antimalarial drug resistance in Africa (68,116). This strategy is composed of three objectives: first, to facilitate the detection of antimalarial drug resistance thereby ensuring a timely response; second, to slow down the emergence of resistance to artemisinin and artemisinin combination therapy (ACT) partner drugs; and third, to reduce the evolution and spread of drug-resistant parasites where resistance has been confirmed (116). The overall goal is to minimize the threat and impact of antimalarial drug resistance due to *P. falciparum* in Africa. Further, four pillars are outlined in the strategy as the pivot for implementation of the objectives: Pillar I seeks to strengthen the surveillance of antimalarial drug efficacy and resistance; pillar II seeks to limit drug pressure through judicious use of malaria diagnostics and therapeutics; pillar III undertakes to limit the spread of antimalarial drug-resistant parasites; and pillar IV looks to stimulate research and innovation into the effective utilization of existing tools in the fight against resistance, as well as to develop novel tools.

Efforts in the surveillance of antimalarial drug resistance are often carried out through population-wide screening or as part of clinical trials. Nyunt *et al*., (2015), showed the presence of resistance in Myanmar, a malaria endemic area in the Greater Mekong subregion of Southeast Asia (117). The study revealed the diversity of *P. falciparum* antimalarial drug resistance genes among local residents in the study region. Mutations in *K13*, *pfarps10*, *pffd*, and *pfmdr2* were identified in the study. Further, mutations in the propeller region of K13 (C580Y) were associated with artemisinin resistance and delayed parasite clearance lasting more than 72 hours after drug administration. Similarly, the V127M, D195Y and T484I mutations were found in the *pfarps10*, *pffd*, and *pfmdr2* genes respectively. In other studies, mutations in *pfcrt* and *pfmdr1* genes were found to be associated with chloroquine resistance in malaria-endemic regions (59,118). In Northern Nigeria, polymorphisms in *pfmdr1* gene were associated with reduced sensitivity to the partner drug lumefantrine, a component of a key ACT drug adopted for use (119). This occurrence was corroborated in Southwest Nigeria, giving hints of parasite drug resistance due to single nucleotide polymorphisms (SNPs) in the *pfmdr1* gene (120).

Prompt diagnosis and treatment:

The WHO GTS (112) advises diagnosis of all suspected malaria cases before administering treatment. Diagnosis through quality-assured microscopy or rapid testing is advised to confirm malaria and thus reduce the over-use of ACTs and the possibility of parasite drug resistance. This practice will especially be useful in malaria endemic settings where a clinical presentation of fever is almost always presumed to be malaria.

Furthermore, prompt and effective treatment of confirmed cases will reduce morbidity and prevent disease progression. *P. vivax* and *P. ovale* spp. confirmed uncomplicated cases should receive a 14-day course of primaquine in addition to ACT, provided they are not pregnant women and are not glucose-6-phosphate dehydrogenase (G6PD) deficient. Parenteral administration of artesunate or artemether should be done in complicated malaria cases, followed by an oral course of ACT. In all treatment cases, oral based monotherapy is discouraged. Intermittent preventive treatment (IPT) of malaria is recommended for pregnant women and infants as these groups are especially vulnerable to malaria. Chemoprophylaxis is likewise recommended for travelers who visit areas of malaria transmission.

Research and development:

Research and development are ongoing for several innovative applications in malaria prophylaxis, diagnosis, and treatment. These include procedures that detect G6PD deficiency to guide primaquine-administered radical cure, vaccines for the most vulnerable, HRP-2 RDT alternatives, and new drugs, among others. In October 2021, the WHO approved use of the RTS,S malaria vaccine for children in moderate to high malaria transmission areas (121). The RTS,S malaria vaccine is an effort of 30 years of research and development by GlaxoSmithKline, supported by various partners. The vaccine roll out is approved for children from 5 months of age, to be received in four doses (122). In addition, the R21/Matrix-M malaria vaccine with over 75% efficacy was recently approved for use in Nigeria (123).

Hemozoin-based assays for diagnosis of malaria are underway with several field tests

carried out (124,125), although none has reached full scale implementation. Similarly, other applications such as saliva and urine-based specimens are currently being explored for the non-invasive diagnosis of malaria (126,127).

New bioactive agents for the treatment of malaria are also in development to cushion the effect of widespread resistance to currently available drugs. Some of these combinations have been dubbed 'Next-generation antimalarials', and include novel products exploring new mechanisms of anti-plasmodial action, and others to be used in combination with current partner drugs. The most currently advanced Next-generation antimalarial agent is the Ganaplacide-lumefantrine combination, which was found to be effective and well tolerated in adult and adolescent patients with uncomplicated *P. falciparum*, following a phase 2 trial (128). In addition, Cipargamin is a phase 2 clinical trial drug that disrupts parasite sodium homeostasis by interfering with the *P. falciparum* ATPase4 (129).

Another Next-generation agent is the M5717-pyronaridine combination which explores the anti-hemozoin activity of pyronaridine, and protein synthesis inhibitory activity of M5717 to achieve anti-plasmodial activity. While pyronaridine (Pyramax) is currently approved for use in an antimalarial regimen, M5717 is a novel agent that targets elongation factor 2 in the protein synthesis pathway of the parasite (130). The M5717-pyronaridine combination is nearing phase 2 clinical trial. Furthermore, adenosine sulfamates, a novel class of nucleoside analogs that inhibit the tyrosine tRNA synthase of the parasite are in preclinical development (131,132). Concerted efforts by the research community, donors and partner agencies will be needed to accelerate research and development towards achieving the 2030 goals of the WHO and associated world organizations.

Contributions of authors:

AEA, FSA and OOA conceptualized the study, AEA reviewed the literature, AVO reviewed the draft, FSA supervised and reviewed the final draft. All authors approved the manuscript submitted for publication.

Source of funding:

No funding was received for the review

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Dagen, M. History of malaria and its treatment. In: Patrick, G. L. (ed). Antimalarial Agents. Chapter 1. Elsevier. 2020: 1–48. https://www.sciencedirect.com/science/article/pii/B9780081012109000019
2. Cunha, C. B., and Cunha, B. A. Brief history of the clinical diagnosis of malaria: From hippocrates to Osler. J Vector Borne Dis. 2008; 45 (3): 194–199.
3. Nosten, F., Richard-Lenoble, D., and Danis, M. A brief history of malaria. Presse Med. 2022; 51 (3): 104130. doi: 10.1016/j.lpm.2022.104130.
4. Arrow, K. J., Panosian, C. B., and Gelband, H. Saving Lives, Buying Time. Saving Lives, Buying Time. Washington, DC: The National Academies Press; 2004: 125–135
5. Talapko, J., Škrlec, I., Alebić, T., Jukić, M., and Včev, A. Malaria: The Past and the Present. Microorganisms. 2019; 7 (6): 179. doi: 10.3390/microorganisms7060179.
6. Neghina, R., Neghina, A. M., Marincu, I., and Iacobiciu, I. Malaria, a journey in time: In search of the lost myths and forgotten stories. Am J Med Sci. 2010; 340 (6): 492–498. http://dx.doi.org/10.1097/MAJ.0b013e3181e7fe6c
7. Brabin, B. J. Malaria's contribution to World War One - The unexpected adversary. Malar J. 2014; 13 (1): 497.doi: 10.1186/1475-2875-13-497
8. Biselli, R., Nisini, R., Lista, F., et al. A Historical Review of Military Medical Strategies for Fighting Infectious Diseases: From Battlefields to Global Health. Biomedicines. 2022; 10 (8): 2050. doi: 10.3390/biomedicines10082050.
9. Shanks, G. Ship-board malaria epidemics during war. BMJ Mil Health. 2021;167 (4): 295–296. doi: 10.1136/bmjmilitary-2021-001873.
10. Laveran, C. L. A., Kean, B. H., Mott, K. E., and Russell, A. J. A newly discovered parasite in the blood of patients suffering from malaria. Parasitic etiology of attacks of malaria. Rev Infect Dis. 1982; 4 (4): 908–911. doi: 10.1093/4.4.908.
11. Cox, F. E. History of the discovery of the malaria parasites and their vectors. Parasit Vectors. 2010; 3 (1): 5. doi: 10.1186/1756-3305-3-5
12. Krotoski, W., Collins, W., Bray, R., et al. Demonstration of hypnozoites in sporozoite-transmitted Plasmodium vivax infection. Am J Trop Med Hyg. 1982; 31: 1291–1293. doi: 10.4269/ajtmh.1982.31.1291.
13. Shortt, H., and Garnham, P. Pre-erythrocytic stages in mammalian malaria parasites. Nature. 1948; 161: 126. doi: 10.1038/161126a0.
14. Garrido-Cardenas, J. A., González-Cerón, L., Manzano-Agugliaro, F., and Mesa-Valle, C. *Plasmodium* genomics : an approach for learning about and ending human malaria. Parasit Res. 2019; 118 (1): 1–27. doi: 10.1007/s00436-018-6127-9
15. Milner DA. Malaria pathogenesis. Cold Spring Harb Perspect Med. 2018; 8 (1): 1–11. doi: 10.1101/cshperspect.a025569.
16. Cowman, A. F., Tonkin, C. J., Tham, W., and Duraisingh, M. T. Review The Molecular Basis of Erythrocyte Invasion by Malaria Parasites. Cell Host Microbe. 2017;22(2):232–45. doi: 10.1016/j.chom.2017.07.003.
17. Zekar, L., and Sharman, T. *Plasmodium falciparum* Malaria. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
18. Menkin-Smith, L., and Winders, W. T. *Plasmodium vivax* Malaria. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
19. Dayananda, K. K., Achur, R. N., and Gowda, D. C. Epidemiology, drug resistance, and pathophysiology of *Plasmodium vivax* malaria. J Vector Borne Dis.

2018; 55 (1) :1–8. doi: 10.4103/0972-9062.234620.
20. Gunalan, K., Lo, E., Hostetler, J. B., et al. Role of *Plasmodium vivax* Duffy-binding protein 1 in invasion of Duffy-null Africans. Proc Natl Acad Sci USA. 2016 ; 113 (22): 6271–6276. doi: 10.1073/pnas.1606113113
21. Ansari, H. R., Templeton, T. J., Subudhi, A. K., et al. Genome-scale comparison of expanded gene families in *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi* with *Plasmodium malariae* and with other *Plasmodium* species. Int J Parasitol 2016; 46 (11): 685–696. doi: 10.1016/j.ijpara.2016.05.009.
22. Stephens, J. W. W. A New Malaria Parasite of Man. Ann Trop Med Parasitol. 1922; 16 (4): 38338–8. https://doi.org/10.1080/00034983.1922.11684331
23. Oguike, M. C., Betson, M., Burke, M., et al. *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* circulate simultaneously in African communities. Int J Parasitol. 2011; 41 (6): 677–683. doi: 10.1016/j.ijpara.2011.01.004.
24. Joste, V., Kamaliddin, C., Kendjo, E., Hubert, V., Argy, N., and Houzé, S. Distinction of *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi* using quantitative Polymerase Chain Reaction with High Resolution Melting revelation. Sci Rep. 2018; 8 (1): 300. https://doi.org/10.1038/s41598-017-18026-1
25. Okafor, C. N., and Finnigan, N. A. *Plasmodium ovale* malaria. In: StatPearls. StatPearls Publishing; 2023.
26. Nolder, D., Oguike, M. C., Maxwell-Scott, H., et al. An observational study of malaria in British travellers: *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi* differ significantly in the duration of latency. BMJ Open. 2013; 3 (5): e002711. doi: 10.1136/bmjopen-2013-002711
27. Rojo-Marcos, G., Rubio-Muñoz, J. M., Ramírez-Olivencia, G., et al. Comparison of imported *Plasmodium ovale curtisi* and *P. ovale wallikeri* infections among patients in Spain, 2005-2011. Emerg Infect Dis. 2014; 20 (3): 409–416. doi: 10.3201/eid2003.130745
28. Yman, V., Wandell, G., Mutemi, D., Miglar, A., Asghar, M., and Hammar, U. Persistent transmission of *Plasmodium malariae* and *Plasmodium ovale* species in an area of declining *Plasmodium falciparum* transmission in eastern Tanzania. PLoS Negl Trop Dis. 2019; 13 (5): e0007414. doi: 10.1371/journal.pntd.0007414
29. Müller, M., and Schlagenhauf, P. *Plasmodium knowlesi* in travellers, update 2014. Int J Infect Dis. 2014; 22: 55–64. doi: 10.1016/j.ijid.2013.12.016.
30. Cuenca, P. R., Key, S., Lindblade, K. A., Vythilingam, I., Drakeley, C., and Fornace, K. Is there evidence of sustained human-mosquito-human transmission of the zoonotic malaria Plasmodium knowlesi? A systematic literature review. Malar J 2022; 21 (1): 89. https://doi.org/10.1186/s12936-022-04110-z
31. Fornace, K. M., Topazian, H. M., Routledge, I., et al. No evidence of sustained nonzoonotic *Plasmodium knowlesi* transmission in Malaysia from modelling malaria case data. Nat Commun. 2023; 14 (1): 2945. https://doi.org/10.1038/s41467-023-38476-8
32. Jeyaprakasam, N., Liew, J., Low, V., Wan-Sulaiman, W-Y., and Vythilingam, I. *Plasmodium knowlesi* infecting humans in Southeast Asia: What's next? PLoS Negl Trop Dis. 2020; 14 (12): e0008900. doi: 10.1371/journal.pntd.0008900.
33. Bettencourt, P. Current challenges in the identification of pre-erythrocytic malaria vaccine candidate antigens. Front Immunol. 2020; 11: 190. doi: 10.3389/fimmu.2020.00190.
34. Zheng, H., Tan, Z., and Xu, W. Immune evasion strategies of pre-erythrocytic malaria parasites. Mediators Inflamm. 2014; 2014: 362605. doi: 10.1155/2014/362605
35. Amino, R., Thiberge, S., Shorte, S., Frischknecht, F., and Ménard, R. Quantitative imaging of *Plasmodium* sporozoites in the mammalian host. C R Biol. 2006; 329 (11): 858–862. doi: 10.1016/j.crvi.2006.04.003
36. Farooq, F., and Bergmann-Leitner, E. S. Immune Escape Mechanisms are *Plasmodium*'s Secret Weapons Foiling the Success of Potent and Persistently Efficacious Malaria Vaccines. Clin Immunol. 2015; 161 (2): 136–143. http://dx.doi.org/10.1016/j.clim.2015.08.015
37. Hopp, C. S., Kanatani, S., Archer, N. K., et al. Comparative intravital imaging of human and rodent malaria sporozoites reveals the skin is not a species-specific barrier. EMBO Mol Med. 2021; 13 (4): 1–18. doi: 10.15252/emmm.201911796
38. Hopp, C. S., Chiou, K., Ragheb, D. R. T., et al. Longitudinal analysis of *Plasmodium* sporozoite motility in the dermis reveals component of blood vessel recognition. Elife. 2015; 4: e07789. https://doi.org/10.7554/eLife.07789
39. Hopp, C. S., and Sinnis, P. The innate and adaptive response to mosquito saliva and *Plasmodium* sporozoites in the skin. Ann N Y Acad Sci. 2015; 1342 (1): 37–43. doi: 10.1111/nyas.12661.
40. Belachew, E. B. Immune response and evasion mechanisms of *Plasmodium falciparum* parasites. J Immunol Res. 2018; 2018: 6529681. doi: 10.1155/2018/6529681
41. Mac-Daniel, L., and Ménard, R. *Plasmodium* and mononuclear phagocytes. Microb Pathog. 2015; 78: 43–51. doi: 10.1016/j.micpath.2014.11.011
42. Boddey, J. A., and Cowman, A. F. *Plasmodium* Nesting : Remaking the Erythrocyte from the Inside Out. Annu Rev Microbiol. 2013; 67: 243–269. doi: 10.1146/annurev-micro-092412-155730.
43. Wunderlich J, Rohrbach P, Dalton JP. The malaria digestive vacuole. Front Biosci (Schol Ed). 2012; 4 (4): 1424–1448. doi: 10.2741/s344.
44. Malaguarnera, L., and Musumeci, S. The immune response to *Plasmodium falciparum* malaria. Lancet Infect Dis. 2002; 2 (8): 472–478. doi: 10.1016/s1473-3099(02)00344-4.
45. de Jong, R. M., Tebeje, S. K., Meerstein-Kessel, L., et al. Immunity against sexual stage *Plasmodium falciparum* and *Plasmodium vivax* parasites. Immunol Rev. 2020; 293 (1): 190–215. doi: 10.1111/imr.12828.
46. Zhang, C., Li, D., Meng, Z., et al. Pyp25α is required for male gametocyte exflagellation. Pathog Dis. 2022; 80 (1): ftac043. doi: 10.1093/femspd/ftac043.
47. Chappell, L., Ross, P., Orchard, L., et al. Refining the transcriptome of the human malaria parasite *Plasmodium falciparum* using amplification-free RNA-seq. BMC Genomics. 2020; 21 (1): 395. doi: 10.1186/s12864-020-06787-5
48. Lee, H. J., Georgiadou, A., Otto, T. D., et al. Transcriptomic studies of malaria: a paradigm for investigation of systemic host-pathogen interactions. Microbiol Mol Biol Rev. 2018; 82 (2): e00071-17. doi: 10.1128/MMBR.00071-17
49. El-Moamly, A. A., and El-Sweify, M. A. Malaria vaccines: the 60-year journey of hope and final success—lessons learned and future prospects. Trop Med Health. 2023; 51: 29. doi: 10.1186/s41182-023-00516-w.
50. Hoffman, S. L., Vekemans, J., Richie, T. L., and Duffy, P. E. The march toward malaria vaccines. Am J Prev Med. 2015; 49 (6 Suppl 4): S319-33. doi: 10.1016/j.amepre.2015.09.011
51. Ibraheem, Z. O., Abd Majid, R., Noor, S. M., Sedik, H. M., and Basir, R. Role of different pf crt and pf mdr-1 mutations in conferring resistance to antimalaria drugs in *Plasmodium falciparum.* Malar

Res Treat. 2014; 2014: 950424. doi: 10.1155/2014/950424
52. Veiga, M. I., Dhingra, S. K., Henrich, P. P., et al. Globally prevalent PfMDR1 mutations modulate *Plasmodium falciparum* susceptibility to artemisinin-based combination therapies. Nat Commun. 2016; 7: 11553. doi: 10.1038/ncomms11553.
53. Shafik, S. H., Richards, S. N., Corry, B., and Martin, R. E. Mechanistic basis for multidrug resistance and collateral drug sensitivity conferred to the malaria parasite by polymorphisms in PfMDR1 and PfCRT. PLoS Biol. 2022; 20: 1–40 http://dx.doi.org/10.1371/journal.pbio.3001616
54. Golassa, L., Kamugisha, E., Ishengoma, D. S., et al. Identification of large variation in pfcrt, pfmdr-1 and pfubp-1 markers in *Plasmodium falciparum* isolates from Ethiopia and Tanzania. Malar J. 2015; 14 (1): 264. doi: 10.1186/s12936-015-0783-3
55. Achol, E., Ochaya, S., Malinga, G. M., Edema, H., and Echodu, R. High prevalence of Pfmdr-1 N86 and D1246 genotypes detected among febrile malaria outpatients attending Lira Regional Referral Hospital, Northern Uganda. BMC Res Notes. 2019; 12 (1): 10–13. https://doi.org/10.1186/s13104-019-4269-1
56. Venkatesan, M., Gadalla, N. B., Stepniewska, K., et al. Polymorphisms in *Plasmodium falciparum* chloroquine resistance transporter and multidrug resistance 1 genes: Parasite risk factors that affect treatment outcomes for *P. falciparum* malaria after artemether-lumefantrine and artesunate-amodiaquine. Am J Trop Med Hyg. 2014; 91 (4): 833–843. doi: 10.4269/ajtmh.14-0031
57. Wicht, K. J., Mok, S., and Fidock, D. A. Molecular mechanisms of drug resistance in *Plasmodium falciparum* malaria. Annu Rev Microbiol. 2020; 74: 431–454. doi: 10.1146/annurev-micro-020518-115546.
58. Baraka, V., Tinto, H., Valea, I., et al. In vivo selection of *Plasmodium falciparum* pfcrt and pfmdr1 variants by artemether-lumefantrine and dihydroartemisinin-piperaquine in Burkina Faso. Antimicrob Agents Chemother. 2015; 59 (1): 734–737. doi: 10.1128/AAC.03647-14
59. Shrivastava, S. K., Gupta, R. K., Mahanta, J., and Dubey, M. L. Correlation of molecular markers, Pfmdr1-N86Y and Pfcrt-K76T, with in Vitro chloroquine resistant *Plasmodium falciparum*, isolated in the malaria endemic States of Assam and Arunachal Pradesh, Northeast India. PLoS One. 2014; 9 (8): e103848. doi: 10.1371/journal.pone.0103848
60. Djimdé, A., Doumbo, O., Cortese, J., et al. A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med. 2001; 344 (4): 257–263. doi: 10.1056/NEJM200101253440403
61. Mohammed, A., Ndaro, A., Kalinga, A., et al. Trends in chloroquine resistance marker, Pfcrt-K76T mutation ten years after chloroquine withdrawal in Tanzania. Malar J. 2013; 12: 415. doi: 10.1186/1475-2875-12-415
62. Nessler, S., Friedrich, O., Bakouh, N., et al. Evidence for activation of endogenous transporters in *Xenopus laevis* oocytes expressing the *Plasmodium falciparum* chloroquine resistance transporter, PfCRT. J Biol Chem. 2004; 279 (38): 39438–39446. doi: 10.1074/jbc.M404671200
63. Shafik, S. H., Cobbold, S. A., Barkat, K., et al. The natural function of the malaria parasite's chloroquine resistance transporter. Nat Commun. 2020; 11: 3922. https://doi.org/10.1038/s41467-020-17781-6
64. Haldar, K., Bhattacharjee, S., and Safeukui, I. Drug resistance in *Plasmodium*. Nat Rev Microbiol. 2018; 16 (3): 156–170. doi: 10.1038/nrmicro.2017.161
65. Mita, T., Tachibana, S. I., Hashimoto, M., and Hirai, M. *Plasmodium falciparum* kelch 13: A potential molecular marker for tackling artemisinin-resistant malaria parasites. Expert Rev Anti Infect Ther. 2016; 14 (1): 125–135. doi: 10.1586/14787210.2016.1106938
66. Xie, S. C., Ralph, S. A., and Tilley, L. K13, the cytostome, and artemisinin resistance. Trends Parasitol. 2020; 36 (6): 533–544. https://doi.org/10.1016/j.pt.2020.03.006
67. Ménard, D., Khim, N., Beghain, J., et al. A Worldwide Map of *Plasmodium falciparum* K13-Propeller Polymorphisms . N Engl J Med. 2016; 374 (25): 2453–2464. doi: 10.1056/NEJMoa1513137.
68. World Health Organization. World malaria report 2022. Geneva, Switzerland; 2022.
69. Aju-Ameh, C. O. Mosquitoes are not the major culprits for the high burden of malaria in Nigeria: a commentary. Pan Afr Med J. 2020; 35: 1–5. doi: 10.11604/pamj.2020.35.11.16972
70. Tadesse, F. G., Slater, H. C., Chali, W., et al. The Relative Contribution of Symptomatic and Asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* Infections to the Infectious Reservoir in a Low-Endemic Setting in Ethiopia. Clin Infect Dis. 2018; 66 (12): 1883–1891. doi: 10.1093/cid/cix1123.
71. Wu, L., Van Den Hoogen, L. L., Slater, H., et al. Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. Nature. 2015; 528 (7580): S86–S93. doi: 10.1038/nature16039.
72. Shekalaghe, S. A., Teun Bousema, J., Kunei, K. K., et al. Submicroscopic *Plasmodium falciparum* gametocyte carriage is common in an area of low and seasonal transmission in Tanzania. Trop Med Int Health. 2007; 12 (4): 547–553. doi: 10.1111/j.1365-3156.2007.01821.x
73. Stresman, G. H., Kamanga, A., Moono, P., et al. A method of active case detection to target reservoirs of asymptomatic malaria and gametocyte carriers in a rural area in Southern Province, Zambia. Malar J. 2010; 9: 265. doi: 10.1186/1475-2875-9-265
74. Ouédraogo, A., Bousema, T., Schneider, P., et al. Substantial contribution of submicroscopical *Plasmodium falciparum* gametocyte carriage to the infectious reservoir in an area of seasonal transmission. PLoS One. 2009; 4: e8410. doi: 10.1371/journal.pone.0008410
75. Churcher, T. S., Bousema, T., Walker, M., et al. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. 2013; 2:e00626. doi: 10.7554/eLife.00626
76. Poti, K. E., Sullivan, D. J., Dondorp, A. M., and Woodrow, C. J. HRP2: Transforming Malaria Diagnosis, but with Caveats. Trends Parasitol. 2020; 36 (2): 112–126. https://doi.org/10.1016/j.pt.2019.12.004
77. Verma, A. K., Bharti, P. K., and Das, A. HRP-2 deletion: a hole in the ship of malaria elimination. Lancet Infect Dis. 2018; 18 (8): 826–827. http://dx.doi.org/10.1016/S1473-3099(18)30420-1
78. Gamboa, D., Ho, M. F., Bendezu, J., et al. A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: Implications for malaria rapid diagnostic tests. PLoS One. 2010; 5 (1): e8091.doi: 10.1371/journal.pone.0008091.
79. Vera-Arias, C. A., Holzschuh, A., Oduma, C. O., et al. High-throughput *Plasmodium falciparum* hrp2 and hrp3 gene deletion typing by digital PCR to monitor malaria rapid diagnostic test efficacy. Elife. 2022; 11: e72083. doi: 10.7554/eLife.72083.
80. Amoah, L. E., Abuaku, B., Bukari, A. H., et al. Contribution of *P. falciparum* parasites with Pfhrp 2

gene deletions to false negative PfHRP 2 based malaria RDT results in Ghana: A nationwide study of symptomatic malaria patients. PLoS One. 2020; 15 (9): e0238749. doi: 10.1371/journal.pone.0238749.

81. Alemayehu, G. S., Blackburn, K., Lopez, K., et al. Detection of high prevalence of *Plasmodium falciparum* histidine-rich protein 2/3 gene deletions in Assosa zone, Ethiopia: implication for malaria diagnosis. Malar J. 2021; 20 (1): 109. doi: 10.1186/s12936-021-03629-x.
82. Funwei, R., Nderu, D., Nguetse, C. N., et al. Molecular surveillance of pfhrp2 and pfhrp3 genes deletion in *Plasmodium falciparum* isolates and the implications for rapid diagnostic tests in Nigeria. Acta Trop. 2019; 196: 121–125. doi: 10.1016/j.actatropica.2019.05.016.
83. Kumari, S., Ahmed, M. Z., Sharma, S., Pande, V., and Anvikar, A. R. Prevalence of Pfhrp2/3 gene deletions among false negative rapid antigen test results in central India. J Vector Borne Dis. 2021; 58 (3): 273–280. doi: 10.4103/0972-9062.328815
84. Thomson, R., Beshir, K. B., Cunningham, J., et al. pfhrp2 and pfhrp3 Gene Deletions That Affect Malaria Rapid Diagnostic Tests for *Plasmodium falciparum*: Analysis of Archived Blood Samples From 3 African Countries. J Infect Dis. 2019; 220 (9): 1444–14452. doi: 10.1093/infdis/jiz335.
85. Gendrot, M., Fawaz, R., Dormoi, J., Madamet, M., and Pradines, B. Genetic diversity and deletion of *Plasmodium falciparum* histidine-rich protein 2 and 3: a threat to diagnosis of *P. falciparum* malaria. Clin Microbiol Infect. 2019; 25 (5): 580–585. doi: 10.1016/j.cmi.2018.09.009
86. Faulde, M. K., Rueda, L. M., and Khaireh, B. A. First record of the Asian malaria vector Anopheles stephensi and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. Acta Trop. 2014; 139: 39–43. http://dx.doi.org/10.1016/j.actatropica.2014.06.016
87. Samarasekera, U. A missed opportunity? *Anopheles stephensi* in Africa. Lancet. 2022; 400 (10367): 1914–1915. doi: 10.1016/S0140-6736(22)02483-7.
88. Seyfarth, M., Khaireh, B. A., Abdi, A. A., Bouh, S. M., and Faulde, M. K. Five years following first detection of *Anopheles stephensi* (Diptera: Culicidae) in Djibouti, Horn of Africa: populations established—malaria emerging. Parasitol Res. 2019; 118 (3): 725–732. doi: 10.1007/s00436-019-06213-0.
89. Balkew, M., Mumba, P., Dengela, D., et al. Geographical distribution of *Anopheles stephensi* in eastern Ethiopia. Parasit Vectors. 2020; 13 (1): 1–8. https://doi.org/10.1186/s13071-020-3904-y
90. Ahmed, A., Khogali, R., Elnour, M. A. B., Nakao, R., and Salim, B. Emergence of the invasive malaria vector *Anopheles stephensi* in Khartoum State, Central Sudan. Parasit Vectors. 2021; 14 (1): 1–5. https://doi.org/10.1186/s13071-021-05026-4
91. USPMI. Strengthening local capacity for the surveillance of Anopheles stephensi in Nigeria. The PMI VectorLink Project. 2023. https://pmivectorlink.org/2023/02/24/strengthening-local-capacity-for-the-surveillance-of-anopheles-stephensi-in-nigeria/
92. Ochomo, E. O., Milanoi, S., Abong'o, B., et al. Detection of *Anopheles stephensi* mosquitoes by molecular surveillance, Kenya. Emerg Infect Dis. 2023; 29 (12): 2498–2508. doi: 10.3201/eid2912.230637
93. Emiru, T., Getachew, D., Teka, H., et al. *Anopheles stephensi* is linked with a recent outbreak of malaria in Dire Dawa City, Ethiopia. In: American Society of Tropical Medicine & Hygiene Meeting. Seattle; 2022. https://plan.core-apps.com/astmh22/abstract/d3220875-3226-4034-bc07-d3fb5bf2170c
94. Yared, S., Gebressielasie, A., Damodaran, L., et al. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. Malar J. 2020; 19 (1): 180. doi: 10.1186/s12936-020-03252-2
95. Balkew, M., Mumba, P., Yohannes, G., et al. An update on the distribution, bionomics, and insecticide susceptibility of *Anopheles stephensi* in Ethiopia, 2018–2020. Malar J. 2021; 20 (1): 1–13. https://doi.org/10.1186/s12936-021-03801-3
96. Thomas, S., Ravishankaran, S., Justin, N. A. J. A., et al. Resting and feeding preferences of *Anopheles stephensi* in an urban setting, perennial for malaria. Malar J. 2017; 16: 111. https://doi.org/10.1186/s12936-017-1764-5
97. Hamlet, A., Dengela, D., Tongren, J. E., et al. The potential impact of *Anopheles stephensi* establishment on the transmission of *Plasmodium falciparum* in Ethiopia and prospective control measures. BMC Med. 2022; 20 (1): 1–10. https://doi.org/10.1186/s12916-022-02324-1
98. Ahn, J., Sinka, M., Irish, S., and Zohdy, S. Modeling marine cargo traffic to identify countries in Africa with greatest risk of invasion by *Anopheles stephensi*. Sci Rep. 2023; 13 (1): 1–12. https://doi.org/10.1038/s41598-023-27439-0
99. Mnzava, A., Monroe, A. C., and Okumu, F. *Anopheles stephensi* in Africa requires a more integrated response. Malar J. 2022; 21: 156. doi: 10.1186/s12936-022-04197-4
100. Gachelin, G., and Opinel, A. Malaria epidemics in Europe after the First World War: The early stages of an international approach to the control of the disease. Hist Cienc Saude Manguinhos. 2011; 18 (2): 431–469. doi: 10.1590/s0104-59702011000200009
101. Gachelin, G., Garner, P., Ferroni, E., Verhave, J. P., and Opinel, A. Evidence and strategies for malaria prevention and control: A historical analysis. Malar J. 2018; 17 (1): 1–18. https://doi.org/10.1186/s12936-018-2244-2
102. World Health Organization. Malaria eradication: benefits, future scenarios and feasibility: a report of the strategic advisory group on malaria eradication. Geneva: 2020. https://www.who.int/publications-detail/strategic-advisory-group-malaria-eradication-executive-summary
103. Maharaj, R., Kissoon, S., Lakan, V., and Kheswa, N. Rolling back malaria in Africa - Challenges and opportunities to winning the elimination battle. S Afr Med J. 2019; 109 (11b): 53–56. doi: 10.7196/SAMJ.2019.v109i11b.14250
104. Myrvang, B., and Godal, T. WHO's malaria program Roll Back Malaria. Tidsskr Nor Laegeforen. 2000; 120 (14): 1661–1664.
105. Narasimhan, V., and Attaran, A. Roll Back Malaria? The scarcity of international aid for malaria control. Malar J. 2003; 2: 1–8. doi: 10.1186/1475-2875-2-8
106. Nabarro, D. Roll Back Malaria. Parassitologia. 1999; 41 (1–3): 501–504.
107. Editorial: Reversing the failures of Roll Back Malaria. Lancet. 2005; 365 (9469): 1439.
108. Yamey, G. Roll Back Malaria: a failing global health campaign. BMJ. 2004; 328 (7448): 1086-1087. doi: 10.1136/bmj.328.7448.1086
109. Editorial: Rolling back malaria—the next 10 years. Lancet. 2008; 372(9645):1193. doi: 10.1016/S0140-6736(08)61494-4
110. Coll-Seck, A. M. A golden age for malaria research and innovation. Malar J. 2008;7 (SUPPL. 1): 1–2. doi 10.1186/1475-2875-7-s1-s2
111. RBMP. The global malaria action plan: for a malaria-free world. 2008. https://www.afro.who.int/sites/default/files/2017-06/Gmapfull.pdf
112. World Health Organization. Global technical strategy

for malaria 2016–2030. Geneva, Switzerland; 2016. https://apps.who.int/iris/bitstream/handle/10665/186671/9789243564999_spa.pdf?sequence=1
113. United Nations. The 17 goals, sustainable development. 2023. https://sdgs.un.org/goals
114. World Health Organization. Vector alert: *Anopheles stephensi* invasion and spread in Africa and Sri Lanka. 2022: 4. https://www.who.int/publications/i/item/9789240067714. (Accessed Aug 10, 2023).
115. Bendix, A., and Alexander, B. New malaria case in Florida brings national total to 8, the first U.S. acquired cases in 20 years. NBC News. 2023. https://www.nbcnews.com/health/health-news/new-malaria-case-florida-brings-national-total-8-first-us-cases-20-yea-rcna94899. (Accessed Oct 28, 2023).
116. World Health Organization. Strategy to respond to antimalarial drug resistance in Africa. Vol. 04. Geneva, Switzerland: World Health Organization; 2022. https://apps.who.int/iris/handle/10665/364531
117. Nyunt, M. H., Shein, T., Zaw, N. N., et al. Molecular evidence of drug resistance in asymptomatic malaria infections, Myanmar, 2015. Emerg Infect Dis. 2017; 23 (3): 517–520. doi: 10.3201/eid2303.161363
118. Andriantsoanirina, V., Ratsimbasoa, A., Bouchier, C., et al. Chloroquine clinical failures in *P. falciparum* malaria are associated with mutant Pfmdr-1, not P fcrt in Madagascar. PLoS One. 2010; 5 (10): e13281 doi: 10.1371/journal.pone.0013281
119. Adamu, A., Jada, M. S., Haruna, H. M. S., et al. *Plasmodium falciparum* multidrug resistance gene-1 polymorphisms in Northern Nigeria: implications for the continued use of artemether-lumefantrine in the region. Malar J. 2020; 19 (1): 1–10. https://doi.org/10.1186/s12936-020-03506-z
120. Dokunmu, T. M., Adjekukor, C. U., Yakubu, O. F., et al. Asymptomatic malaria infections and Pfmdr1 mutations in an endemic area of Nigeria. Malar J. 2019; 18 (1): 1–7. https://doi.org/10.1186/s12936-019-2833-8
121. World Health Oragnization. WHO recommends groundbreaking malaria vaccine for children at risk. 2021. https://www.who.int/news/item/06-10-2021-who-recommends-groundbreaking-malaria-vaccine-for-children-at-risk (Accessed Aug 22, 2023).
122. Willyard, C. The next frontier for malaria vaccination. Nature. 2023; 618 (7967): S20–S22. doi: 10.1038/d41586-023-02048-z.
123. Reuters. Nigeria regulator grants approval to Oxford's malaria vaccine. Reuters. 2023 https://www.reuters.com/world/africa/nigeria-regulator-grants-approval-oxfords-malaria-vaccine-2023-04-17/. (Accessed Aug 23, 2023).
124. Baptista, V., Peng, W. K., Minas, G., Veiga, M. I., and Catarino, S. O. Review of Microdevices for Hemozoin-Based Malaria Detection. Biosensors (Basel). 2022; 12 (2): 110. doi: 10.3390/bios12020110
125. de Melo, G. C., Netto, R. L. A., Mwangi, V. I., et al. Performance of a sensitive haemozoin-based malaria diagnostic test validated for vivax malaria diagnosis in Brazilian Amazon. Malar J. 2021; 20 (1): 146. https://doi.org/10.1186/s12936-021-03688-0
126. Chai, H. C., and Chua, K. H. Urine and saliva: relevant specimens for malaria diagnosis? Diagnostics (Basel). 2022; 12 (12): 2989. doi: 10.3390/diagnostics12122989.
127. Aninagyei, E., Abraham, J., Atiiga, P., Antwi, S. D., Bamfo, S., and Acheampong, D. O. Evaluating the potential of using urine and saliva specimens for malaria diagnosis in suspected patients in Ghana. Malar J. 2020; 19 (1): 349. doi: 10.1186/s12936-020-03427-x.
128. Ogutu, B., Yeka, A., Kusemererwa, S., et al. Ganaplacide (KAF156) plus lumefantrine solid dispersion formulation combination for uncomplicated *Plasmodium falciparum* malaria: an open-label, multicentre, parallel-group, randomised, controlled, phase 2 trial. Lancet Infect Dis. 2023; 23 (9): 1051-1061. https://doi.org/10.1016/S1473-3099(23)00209-8
129. Schmitt, E. K., Ndayisaba, G., Yeka, A., et al. Efficacy of Cipargamin (KAE609) in a Randomized, Phase II Dose-Escalation Study in Adults in Sub-Saharan Africa With Uncomplicated *Plasmodium falciparum* Malaria. Clin Infect Dis 2022; 74 (10): 1831–1839. doi: 10.1093/cid/ciab716
130. Rottmann, M., Jonat, B., Gumpp, C., et al. Preclinical Antimalarial Combination Study of M5717, a *Plasmodium falciparum* Elongation Factor 2 Inhibitor, and Pyronaridine, a Hemozoin Formation Inhibitor. Antimicrob Agents Chemother. 2020; 64 (4): e02181-19. doi: 10.1128/AAC.02181-19.
131. Sundararaman, S. A., and John, A. R. Adenosine sulfamates: Next generation of antimalarials. Cell Host Microbe. 2022; 30 (8): 1074–1076. doi: 10.1016/j.chom.2022.07.007
132. Xie, S. C., Metcalfe, R. D., Dunn, E., et al. Reaction hijacking of tyrosine tRNA synthetase as a new whole-of-life-cycle antimalarial strategy. Science. 2022; 376 (6597): 1074–1079. doi: 10.1126/science.abn0611.

Sawadogo et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 263 - 268 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2491. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Burden and predictors of drug-resistant tuberculosis in Burkina Faso

[1]Sawadogo, T. L., *[1]Yooda, A. P., [2]Kambiré, D., [3]Simporé, A., [4]Nagalo, Y. A., [5]Diarra, A., [2]Zoure, A., A., [1]Diallo, A., [1]Paré, S. J. E., [1]Combary, A., [1]Saouadogo, T., [6]Sanou, G., [7]Sanou, A. M., [6]Bonkoungou, I. J. O., and [2]Diabouga, P. S.

[1]Programme National de Lutte contre la Tuberculose, Ministère de la Santé, Ouagadougou, Burkina Faso
[2]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), LR-Maladies Infectieuses et Parasitaires (LR-MIP), Ouagadougou, Burkina Faso
[3]Agence Nationale Pour la Sécurité Sanitaire de l'Environnement, de l'Alimentation, du Travail et des Produits de Santé (ANSSEAT), Ouagadougou, Burkina Faso
[4]Université Lédéa Bernard Ouédraogo, Unité de Formation et de Recherche en Sciences de la Santé, Ouahigouya, Burkina Faso
[5]Groupe de Recherche Action en Santé (GRAS)
[6]Université Joseph KI ZERBO/ Unité de Génomique des pathogènes One-Health, Ouagadougou, Burkina Faso
[7]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), LR-Maladies Infectieuses et Parasitaires (LR-MIP), BOBO Dioulasso, Burkina Faso
*Correspondence to: paulyooda@gmail.com

Abstract:

Background: In Burkina Faso, the emergence of resistance within the *Mycobacterium tuberculosis* complex (MTBC) represents a growing public health challenge, threatening the progress made toward tuberculosis (TB) control. This study aimed to estimate the prevalence of rifampicin- and isoniazid-resistant TB and to identify factors associated with multidrug-resistant TB (MDR-TB) among patients managed at the National Tuberculosis Control Center (CNLAT).
Methodology: A cross-sectional study was conducted using samples from 3,485 presumptive and confirmed TB patients received at CNLAT from January 2020 to December 2023. MTBC detection and rifampicin resistance screening were performed using the Xpert MTB/RIF assay. Samples testing rifampicin-resistant were further analyzed with the GenoType MDR*plus* v2.0 assay to assess concurrent resistance to isoniazid and rifampicin.
Results: The overall prevalence of rifampicin-resistant TB (RR-TB) among 3,485 TB patients was 2.7% (95% CI: 2.2–3.3), 1.9% among new cases and 11.7% among previously treated cases. Of 60 RR-TB cases identified by Xpert MTB/RIF assay tested by GenoType MDR*plus*, 73.3% (44/60) were confirmed multidrug-resistant (MDR-TB). Significant associations were observed between MDR/RR-TB and age (p=0.044) and previous TB treatment history (p<0.001).
Conclusion: This study reveals a notable burden of MDR/RR-TB (2.7%) in Burkina Faso, emphasizing the need for systematic molecular testing and robust patient monitoring throughout treatment. Strengthening early diagnosis, treatment adherence, and molecular surveillance will be critical to limiting the spread of drug-resistant TB and achieving national TB elimination targets.

Keywords: Multidrug-resistant tuberculosis, rifampicin resistance, molecular diagnostics, surveillance, Burkina Faso

Received Feb 22, 2026; Revised Mar 10, 2026; Accepted Mar 11, 2026

Charge et facteurs prédictifs de la tuberculose pharmacorésistante au Burkina Faso

[1]Sawadogo, T. L., *[1]Yooda, A. P., [2]Kambiré, D., [3]Simporé, A., [4]Nagalo, Y. A., [5]Diarra, A., [2]Zoure, A., A., [1]Diallo, A., [1]Paré, S. J. E., [1]Combary, A., [1]Saouadogo, T., [6]Sanou, G., [7]Sanou, A. M., [6]Bonkoungou, I. J. O., et [2]Diabouga, P. S.

[1]Programme National de Lutte contre la Tuberculose, Ministère de la Santé, Ouagadougou, Burkina Faso

[2]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), LR-Maladies Infectieuses et Parasitaires (LR-MIP), Ouagadougou, Burkina Faso
[3]Agence Nationale Pour la Sécurité Sanitaire de l'Environnement, de l'Alimentation, du Travail et des Produits de Santé (ANSSEAT), Ouagadougou, Burkina Faso
[4]Université Lédéa Bernard Ouédraogo, Unité de Formation et de Recherche en Sciences de la Santé, Ouahigouya, Burkina Faso
[5]Groupe de Recherche Action en Santé (GRAS)
[6]Université Joseph KI ZERBO/ Unité de Génomique des pathogènes One-Health, Ouagadougou, Burkina Faso
[7]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), LR-Maladies Infectieuses et Parasitaires (LR-MIP), BOBO Dioulasso, Burkina Faso
*Correspondence à: paulyooda@gmail.com

Résumé:

Contexte: Au Burkina Faso, l'émergence de résistances au sein du complexe Mycobacterium tuberculosis (MTBC) représente un défi croissant de santé publique, menaçant les progrès réalisés dans la lutte contre la tuberculose (TB). Cette étude visait à estimer la prévalence de la TB résistante à la rifampicine et à l'isoniazide et à identifier les facteurs associés à la tuberculose multirésistante (TB-MR) chez les patients pris en charge au Centre national de lutte contre la tuberculose (CNLAT).
Méthodologie: Une étude transversale a été menée à partir d'échantillons prélevés chez 3 485 patients présentant une tuberculose présumée ou confirmée, admis au CNLAT entre janvier 2020 et décembre 2023. La détection du MTBC et le dépistage de la résistance à la rifampicine ont été réalisés à l'aide du test Xpert MTB/RIF. Les échantillons résistants à la rifampicine ont ensuite été analysés par le test GenoType MDRplus v2.0 afin d'évaluer la résistance concomitante à l'isoniazide et à la rifampicine.
Résultats: La prévalence globale de la tuberculose résistante à la rifampicine (TB-RR) parmi 3485 patients tuberculeux était de 2,7% (IC à 95%: 2,2–3,3), soit 1,9% parmi les nouveaux cas et 11,7% parmi les cas déjà traités. Sur 60 cas de TB-RR identifiés par le test Xpert MTB/RIF et confirmés par GenoType MDRplus, 73,3% (44/60) étaient des cas confirmés de tuberculose multirésistante (TB-MR). Des associations significatives ont été observées entre la TB-MR/RR et l'âge (p=0,044) ainsi que les antécédents de traitement antituberculeux (p<0,001).
Conclusion: Cette étude révèle une prévalence importante de TB-MR/RR (2,7%) au Burkina Faso, soulignant la nécessité de tests moléculaires systématiques et d'un suivi rigoureux des patients tout au long du traitement. Le renforcement du diagnostic précoce, de l'observance thérapeutique et de la surveillance moléculaire sera essentiel pour limiter la propagation de la tuberculose résistante aux médicaments et atteindre les objectifs nationaux d'élimination de la tuberculose.

Mots-clés: Tuberculose multirésistante, résistance à la rifampicine, diagnostic moléculaire, surveillance, Burkina Faso

Introduction:

Tuberculosis (TB) remains one of the leading causes of infectious morbidity and mortality worldwide, despite significant advances in diagnosis and treatment. The emergence and spread of drug-resistant forms of the *Mycobacterium tuberculosis* complex (MTBC) represent a major threat to global public health and a critical obstacle to achieving the World Health Organization (WHO) End TB Strategy targets for 2035 (1). Resistance to first-line anti-tuberculosis drugs, particularly rifampicin and isoniazid, compromises the efficacy of standard treatment regimens and increases the risk of treatment failure, relapse, and community transmission of resistant strains (2-5).

Globally, the WHO estimates that more than 10 million people continue to fall ill with TB each year, resulting in over one million deaths (5). Between 2020 and 2023, the annual number of individuals who developed rifampicin-resistant (RR-TB) or multidrug-resistant TB (MDR-TB) remained relatively stable, representing approximately 3–4% of new TB cases and 16–17% of previously treated cases (1,5). The highest proportions of MDR- and XDR-TB cases were reported in the Russian Federation and in several countries across Eastern Europe and Central Asia (5). According to the five most recent WHO global TB reports, Africa remains one of the three WHO regions most heavily affected by the disease (1,5). Within the continent, Nigeria, the Republic of the Congo, and South Africa are among the 30 countries with the highest TB burden (1,5).

In Burkina Faso, TB continues to pose a serious public health concern. The latest national drug resistance surveillance survey, conducted in 2019, reported rifampicin resistance in 2.0% of new cases and 14.5% of previously treated cases, 83% of which were classified as MDR-TB(6). However, since this survey, limited updated data have been available, and disruptions related to the COVID-19 pandemic may have further impacted TB detection, treatment adherence, and resistance dynamics.

Given the clinical and epidemiological implications of multidrug-resistant TB, continuous and up-to-date surveillance is essential to inform diagnostic and therapeutic strategies. This study, therefore aimed to estimate the prevalence of rifampicin-resistant and multi-drug-re-

sistant TB (RR/MDR-TB) among patients' managed at the National Tuberculosis Control Center (CNLAT) in Burkina Faso between 2020 and 2023, thereby contributing to the national update of anti-TB drug resistance surveillance data.

Materials and method:

Study setting:

This study was conducted at the National Tuberculosis Control Center (CNLAT), located in Ouagadougou, in the central region of Burkina Faso. The CNLAT hosts the National Reference Laboratory for Mycobacteria (LNR-M), which serves as the country's main facility for TB diagnosis, drug susceptibility testing, and molecular surveillance.

Study design and period:

A descriptive cross-sectional study was carried out over four years, from January 1, 2020, to December 31, 2023. This timeframe was selected to capture a representative overview of the evolution of drug resistance patterns in the MTBC during the post-COVID-19 period.

Study population:

The study included all patients with suspected or confirmed tuberculosis whose biological samples were received at the CNLAT for bacteriological confirmation and drug resistance testing. Patients originated from multiple regions across Burkina Faso, encompassing both new TB cases and previously treated cases.

Ethical considerations:

The study received approval from the Burkina Faso Health Research Ethics Committee (deliberation n° 2015-6-080). Patient confidentiality and anonymity were strictly maintained throughout the study in accordance with ethical standards for biomedical research.

Collection of biological samples:

Biological samples varied according to the suspected form of TB. For pulmonary TB, early-morning sputum specimens (≥5 mL) were collected by patients into sterile containers while for extrapulmonary TB, ascitic fluid, lymph node pus, pleural fluid, gastric aspirates, or bronchoalveolar lavage were collected aseptically by trained medical personnel.

Samples were transported to the LNR-M either by patients, accompanying staff, or through the Integrated Biological Sample Transport System (SITEB) within 48 hours. When immediate transport was not feasible, samples were refrigerated at +2°C to +8°C and shipped within a maximum of 48 hours.

Detection of the *Mycobacterium tuberculosis* complex and rifampicin resistance:

Detection of MTBC and rifampicin resistance was performed simultaneously using the Xpert MTB/RIF assay (Cepheid®, Sunnyvale, USA). This real-time PCR-based test targets the *rpoB* gene, identifying mutations within the Rifampicin Resistance Determining Region (RRDR, codons 505–533). Results were interpreted according to the manufacturer's guidelines and the National Tuberculosis Control Programme (PNLT) standards.

Detection of isoniazid resistance:

Isoniazid resistance testing was conducted on samples showing rifampicin resistance using the GenoType® MDR*plus* v2.0 Line Probe Assay (Hain Lifescience GmbH, Germany), following WHO-recommended protocols. This reverse hybridization assay detects mutations in *rpoB* gene (codons 505–533) for rifampicin resistance, and *katG* gene (codon 315) and *inhA* promoter for isoniazid resistance. Resistance profiles were determined based on the hybridization pattern of amplified DNA fragments to mutation-specific probes.

Data analysis:

Data were entered and cleaned using Microsoft Excel 2016, then analyzed with R software version 4.4.1. Descriptive statistics summarized baseline characteristics. Associations between independent variables (age, sex, treatment history, TB type) and resistance outcomes were assessed using Chi-square tests and univariate and multivariate logistic regression analyses. A p value < 0.05 was considered statistically significant.

Results:

Sociodemographic and clinical characteristics of the study population:

A total of 3,485 tuberculosis patients were included in the study, of whom 92.1% (n=3,211) were new cases, and 7.9% (n=274) had been previously treated. The participants' ages ranged from 1 to 99 years, with a mean age of 38 ± 5.8 years. Males accounted for the majority of cases (77.4%) compared with females (22.6%), with a male-to-female ratio of approximately 3.4:1. The most represented age group was 25–44 years, comprising 51.7% of the patients.

Table 1: Socio-demographic characteristics of the patients with suspected or confirmed tuberculosis at the National Tuberculosis Control Center (CNLAT), Ouagadougou, Burkina Faso

Characteristics	Number (n = 3,485)	Percentage (%)
Gender		
Male	2,698	77.4
Female	787	22.6
Age group (years)		
0–14	90	2.6
15–24	437	12.5
25–34	904	25.9
35–44	899	25.8
45–54	561	16.1
55–64	325	9.3
≥ 65	269	7.7
Treatment history		
New cases	3,211	92.1
Previously treated	274	7.9
Type of tuberculosis		
Pulmonary	3,388	97.2
Extrapulmonary	97	2.8

Most cases were pulmonary tuberculosis (97.2%), while extrapulmonary forms represented only 2.8% of diagnoses (Table 1). This demographic pattern reflects the typical profile of TB patients in high-burden settings, where young adult males, the most economically active segment of the population, are disproportionately affected.

Prevalence and risk factors associated with rifampicin resistance:

Among 3,485 tuberculosis cases confirmed by the Xpert MTB/RIF test, 94 rifampicin-resistant TB (RR-TB) cases were detected, corresponding to an overall prevalence of 2.7%. Of these, 1.9% (62/3,211) occurred among new cases, and 11.7% (32/274) among previously treated patients.

In univariate analysis, age group and treatment history were significantly associated with RR-TB. Adult patients aged 25–44 years had a higher likelihood of developing RR-TB (OR = 2.47; 95% CI: 1.25–5.60; $p = 0.017$), which remained significant in multivariate analysis (adjusted OR = 2.18; 95% CI: 1.08–5.01; $p = 0.044$).

Similarly, patients with a history of TB treatment showed a strong association with RR-TB (adjusted OR = 6.33; 95% CI: 3.99–9.87; $p < 0.001$). No significant association was found with sex ($p = 0.633$) or other age categories (Table 2). These findings indicate that previous TB treatment and adult age (25–44 years) are major determinants of rifampicin resistance in this population.

Table 2: Prevalence and risk factors associated with rifampicin resistance in the tuberculosis

Variable	Total (n)	RR-TB prevalence (%)	Univariate OR (95% CI)	*p* value	Multivariate OR (95% CI)	*p* value
Gender						
Male	2,698	2.78	1.16 (0.71–1.98)	0.578	0.88 (0.53–1.53)	0.633
Female	787	2.41	Ref	—	Ref	—
Age group (years)						
0–24	904	3.2	Ref	—	Ref	—
25–44	899	4.1	2.47 (1.25–5.60)	0.017	2.18 (1.08–5.01)	0.044
45–64	561	1.8	1.35 (0.60–3.30)	0.489	1.25 (0.55–3.09)	0.614
≥ 65	269	0.7	0.49 (0.07–1.96)	0.363	0.48 (0.07–1.94)	0.354
Treatment history						
New cases	3,211	1.93	Ref	—	Ref	—
Previously treated	274	11.68	6.72 (4.25–10.42)	<0.001	6.33 (3.99–9.87)	0.001

Table 3: Proportion of *Mycobacterium tuberculosis* complex resistant to rifampicin and isoniazid using Xpert MTB/RIF and GenoType MDR*plus* v2.0

Tests	Drug	Total (N)	New cases (Resistant/Total)	%	Previously treated (Resistant/Total)	%	Overall resistance (%)
Xpert MTB/RIF	RIF	3,485	62/3,211	1.9	32/274	11.68	2.7
GenoType MDRplus v2.0	INH	60	34/46	73.9	10/14	71.4	73.3
GenoType MDRplus v2.0	RIF	60	46/46	100	14/14	100	100
Combined (INH + RIF)	—	60	34/46	73.9	10/14	71.4	73.3

RIF = Rifampicin; INH = Isoniazid

Resistance profile of the *Mycobacterium tuberculosis* complex:

Among 60 RR isolates from Xpert MTB/RIF assay tested with GenoType MDR*plus* v2.0, all were confirmed resistant to rifampicin, and 44 (73.3%) were also resistant to isoniazid, meeting the definition of multidrug-resistant TB (MDR-TB). The MDR-TB prevalence was comparable between new (73.9%) and previously treated cases (71.4%) (Table 3).

Discussion:

This study aimed to estimate the prevalence and associated factors of multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) among patients diagnosed at the National Tuberculosis Control Center (CNLAT) in Burkina Faso. The overall prevalence of MDR/XDR-TB was 2.7%, including 1.9% among new cases and 11.7% among previously treated cases. Among rifampicin-resistant isolates, 73.3% also exhibited isoniazid resistance, confirming MDR-TB.

The prevalence observed in this study is comparable to that reported in Ghana (2.4%) (7), but lower than that documented in Ethiopia (5.3%) (11), Botswana (5.4%) (9), and Nigeria (5.8%) (10), illustrating the heterogeneous geographical distribution of MDR/XDR-TB across Africa. These results place Burkina Faso among countries with a relatively low prevalence (<3%), consistent with the WHO 2024 global estimate of 3.2% (6). The similarity between our findings and those of the 2019 national resistance survey (2.0%) (7) suggests a stable trend in drug resistance in Burkina Faso over the past five years.

However, the higher prevalence of MDR/XDR-TB among previously treated patients (11.7%) underscores the need to strengthen treatment adherence and follow-up. This prevalence is slightly lower than that reported in Burkina Faso (14.5%) in 2019 (7) and below the global prevalence (16%) reported by WHO in 2024 (6). Similar findings have been observed in other African countries such as Ghana (1.3% vs 25%) (8), Ethiopia (2.3% vs 14.36%) (11), Nigeria (6.0% vs 32%) (10), and Botswana (1.3% vs 7.7%) (9). These differences confirm that previous treatment remains a major determinant of drug resistance, as shown by our results (aOR=6.33; 95% CI: 3.99–9.87; p<0.001), consistent with earlier studies in Burkina Faso (14,15).

Inadequate drug regimens, poor-quality medications, suboptimal prescription practices, and poor adherence (2,5,14) contribute to the development of resistance through selective pressure on *M. tuberculosis* strains. In addition, the study revealed that patients aged 25–44 years were more likely to develop MDR/XDR-TB (aOR=2.18; 95% CI: 1.08–5.01; p=0.044). This association was not observed in the 2019 national survey (7) but could be related to behavioural and occupational factors, as this age group represents the most active and mobile segment of the population. Frequent work-related mobility may compromise treatment adherence, favouring the emergence of resistant forms. Further studies are needed to explore socio-behavioural determinants of resistance in this group. Conversely, no significant association was found between gender and MDR/XDR-TB, consistent with previous studies conducted in Burkina Faso (8,15).

Regarding the resistance profile, 73.3% of rifampicin-resistant strains were also resistant to isoniazid, which aligns with previous national data from 2017 showing approximately 83% dual resistance (7). This observation confirms that in Burkina Faso, the majority of RR-TB cases correspond to MDR-TB. Globally, several studies have reported that rifampicin monoresistance is rare, as resistance to rifampicin is frequently associated with isoniazid resistance in about 75% of cases (2,3,16). For this reason, rifampicin resistance is widely used as a surrogate marker for MDR-TB, justifying the immediate initiation of MDR-TB treatment upon RR-TB detection (13).

Conclusion:

This study provides updated insights into the burden and determinants of drug-resistant tuberculosis in Burkina Faso, revealing a moderate but persistent prevalence of MDR/

XDR-TB. The findings show that resistance remains significantly associated with previous TB treatment and with the 25–44-year-old age group, suggesting that both therapeutic history and population mobility play a crucial role in the emergence of resistant forms.

To effectively contain the spread of MDR/XDR-TB, it is crucial to strengthen molecular diagnostic capacity and ensure the rapid and decentralized detection of resistant cases. Continuous monitoring of treatment adherence and improvement of directly observed therapy are essential to prevent therapeutic failure and relapse. Furthermore, expanding surveillance of drug resistance and integrating genomic tools such as whole genome sequencing will improve the early detection of transmission clusters and the understanding of circulating resistant strains.

Sustained investment in diagnostic infrastructure, health worker training, and patient education is indispensable to improving therapeutic outcomes and interrupting transmission chains. These actions are vital for achieving the WHO End TB Strategy targets for 2035 and for preventing the further expansion of drug-resistant tuberculosis in Burkina Faso and across the region.

Contributions of authors:

APY, TLS, YAN, and DK conceived and designed the study; APY, TLS, YAN, DK, AAZ, AD, AS, AmD, SJEP, and MAS were involved in data acquisition and drafting the manuscript; APY, AS, and GS performed the statistical analysis and interpretation; and TS, AC, IJOB and, and PSD approved the final version of the manuscript.

Source of funding:

This study did not receive any funding from a funding organization.

Conflict of interest:

Authors declared no conflict of interest.

References:

1. World Health Organization. Global Tuberculosis https://apps.who.int/iris/bitstream/handle/10665/353790/9789240041622-fre.pdf?sequence=1 (Accessed 25 Jan 2025).
2. Noeske, J., and Nguenko, P. N. Impact of resistance to anti-tuberculosis drugs on treatment outcome using World Health Organization standard regimen Trans R Soc Trop Med Hyg. 2002; 96 (4): 429-433. doi: 10.1016/s0035-9203(02)90383-4
3. Coovadia, Y. M., Mahomed, S., Pillay, M., Werner, L and Mlisana, K. Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. PLoS One. 2013; 8 (11): e77712. doi: 10.1371/journal.pone.0077712
4. Tegegne, K. T., Zenebe, A., Assefa, A. A., et al. Determinants of Multidrug Resistance Tuberculosis: Systemic Review and Meta-analysis. Acta Sci Cancer Biol. 2021; 5: 2.
5. Lin, S., Wei, S., Zhao, Y., Dai, Z., Lin, J., and Pang, Y. Genetic Diversity and Drug Susceptibility Profiles of Multidrug-Resistant Tuberculosis Strains in Southeast China. Infect Drug Resist. 2021; 14: 3979-3989. doi: 10.2147/IDR.S331516
6. World Health Organization. Global tuberculosis report 2024. World Health Organization; 2024: 69.
7. Diandé, S., Badoum, G., Combary, A., et al. Multidrug-resistant tuberculosis in Burkina Faso from 2006 to 2017: results of national surveys. Eur J Microbiol Immunol. 2019; 9 (1): 23-28. doi: 10.1556/1886.2018.00029
8. Sylverken, A. A., Kwarteng, A., Twumasi-Ankrah, S., et al. The burden of drug resistance tuberculosis in Ghana; results of the First National Survey. PLoS One. 2021; 16 (6): e0252819. https://doi.org/10.1371/journal.pone.0252819
9. Tembo, B. P., and Malangu, N. G. Prevalence and factors associated with multidrug/rifampicin resistant tuberculosis among suspected drug-resistant tuberculosis patients in Botswana. BMC Infect Dis. Dec 2019; 19 (1): 779. doi: 10.1186/s12879-019-4375-7
10. Olaosebikan, V. O., Manga, S. B., Danladi, Y. K., Udefi, A. C., and Adedokun, A. I. Prevalence of rifampicin-resistant *Mycobacterium tuberculosis* in Kebbi state, Nigeria. Eur J Biol Res. 2022; 12 (4): 320-329. doi: 10.5281/zenodo.7420049
11. Ejeta, E., Beyene, G., Bonsa, Z., and Abebe, G. Xpert MTB/RIF assay for the diagnosis of *Mycobacterium tuberculosis* and Rifampicin resistance in high human immunodeficiency virus setting in Gambella regional state, southwest Ethiopia. J Clin Tuberc Mycobact Dis. 2018; 12: 14-20. doi: 10.1016/j.jctube.2018.06.002
12. Kateregga, N. J., Atuheire, C., Ntambi, S., Ocaka, D., Ndukui, G. J., and Wampande, E. Prevalence of multidrug resistant *Mycobacterium tuberculosis* and risk factors among youth attending MDR-TB unit in Mulago hospital. J Bacteriol Mycol. 2018; 5 (9): 1088.
13. Vareldzis, B. P., Grosset, J., De Kantor, I., et al. Drug-resistant tuberculosis: laboratory issues: World Health Organization recommendations. Tuber Lung Dis. 1994; 75 (1): 1-7. doi: 10.1016/0962-8479(94)90096-5
14. Diandé, S., Sangaré, L., Kouanda, S., Dingtoumda, B. I., and Traoré, A. S. Drug resistance of *Mycobacterium tuberculosis* complex among newly diagnosed tuberculosis cases in Burkina Faso. West Afr J Med 2009; 28 (6): 353-357 doi: 10.4314/wajm.v28i6.55020
15. Sangaré, L., Diandé, S., Badoum, G., Dingtoumda, B., and Traoré, A. S. Anti-tuberculosis drug resistance in new and previously treated pulmonary tuberculosis cases in Burkina Faso. Int J Tuberc Lung Dis. 2010; 14 (11): 1424-1429.
16. Bedaso, M. H., and Kalil, F. S. Trends of Drug Resistance Tuberculosis from 2014 to 2018, Bale Zone, Oromia Region, Ethiopia. Infect Drug Resist. 2021; 14: 2073-2078. doi: 10.2147/IDR.S300723

Irek et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 269 - 276 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2491. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Bridging diagnostic gaps of drug-resistant pulmonary tuberculosis with clinical diagnosis in a low-and middle-income state, southwest Nigeria: A secondary data analysis

*[1]Irek, E. O., [2]Isinkaye, A. O., and [3]Orojo, J. A.

[1]Department of Medical Microbiology and Parasitology, Afe Babalola University/Afe Babalola University Multi-System Hospital, Ado-Ekiti, Nigeria
[2]Department of Community Medicine, Federal Teaching Hospital, Ido, Ekiti, Nigeria
[3]Tuberculosis Leprosy and Buruli Control Programme, Primary Health Care Development Agency, Ado-Ekiti, Nigeria
*Correspondence to: dj1irek@yahoo.com; +2348136833485; ORCID: 0000-0001-6363-6645

Abstract:

Background: Drug-resistant tuberculosis (DR-TB) poses a significant challenge to global TB control efforts, particularly in low- and middle-income countries (LMICs) where diagnostic resources are limited, thereby widening treatment gap. This has increased morbidity and mortality due to TB in Sub-Saharan Africa. We sought to evaluate the role of clinical diagnosis in identifying DR-TB in pulmonary TB cases missed by the sputum GeneXpert MTB/RIF assay in Ekiti State, Nigeria.
Methodology: A secondary data analysis was conducted using the 2024 National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP) register in Ekiti State. Data on demographics, diagnostic methods, and resistance patterns were extracted and analyzed using descriptive statistics.
Results: Among 28 DR-TB cases identified, GeneXpert MTB/RIF assay detected only 53.6% (n=15), missing 46.4% (n=13) which were subsequently identified through clinical suspicion followed by Line Probe Assay. MDR-TB accounted for 46.4% (n=13), rifampicin mono-resistance for 39.3% (n=11), and isoniazid mono-resistance for 14.3% (n=4). Two cases of pre-XDR TB were observed among people living with HIV, both of whom had false-negative GeneXpert results. The average diagnostic delay from GeneXpert testing to LPA-based diagnosis was 16 days.
Conclusion: Clinical evaluation is critical in bridging diagnostic gaps created by false-negative GeneXpert results, ensuring timely identification and treatment of DR-TB.

Keywords: Diagnostic gaps, Nigeria, GeneXpert, Tuberculosis

Received Jan 29, 2026; Revised Mar 2, 2026; Accepted Mar 4, 2026

Combler les lacunes diagnostiques de la tuberculose pulmonaire pharmacorésistante par le diagnostic clinique dans un État à revenu faible et intermédiaire du sud-ouest du Nigéria: Une analyse de données secondaires

*[1]Irek, E. O., [2]Isinkaye, A. O., et [3]Orojo, J. A.

[1]Département de Microbiologie Médicale et de Parasitologie, Université Afe Babalola/Hôpital Universitaire Multisystème Afe Babalola, Ado-Ekiti, Nigéria
[2]Département de Médecine Communautaire, Hôpital Universitaire Fédéral, Ido, Ekiti, Nigéria
[3]Programme de Lutte Contre la Tuberculose, la Lèpre et le Virus Buruli, Agence de Développement des Soins de Santé Primaires, Ado-Ekiti, Nigéria
*Correspondance à: dj1irek@yahoo.com; +2348136833485 ORCID: 0000-0001-6363-6645

Résumé:

Contexte: La tuberculose pharmacorésistante (TB-DR) représente un défi majeur pour la lutte mondiale contre la tuberculose, en particulier dans les pays à revenu faible et intermédiaire (PRFI) où les ressources diagnostiques sont limitées, ce qui creuse l'écart de traitement. Cette situation a entraîné une augmentation de la morbidité et de la mortalité liées à la tuberculose en Afrique subsaharienne. Nous avons cherché à évaluer le rôle du diagnostic clinique dans l'identification de la TB-DR chez les patients atteints de tuberculose pulmonaire non détectés par le test GeneXpert MTB/RIF sur expectorations dans l'État d'Ekiti, au Nigéria.
Méthodologie: Une analyse secondaire des données a été réalisée à partir du registre 2024 du Programme national de lutte contre la tuberculose, la lèpre et l'ulcère de Buruli (NTBLCP) de l'État d'Ekiti. Les données démographiques, les méthodes diagnostiques et les profils de résistance ont été extraits et analysés à l'aide de statistiques descriptives.
Résultats: Parmi les 28 cas de tuberculose pharmacorésistante (TB-DR) identifiés, le test GeneXpert MTB/RIF n'en a détecté que 53,6 % (n = 15), manquant ainsi 46,4 % des cas (n = 13), qui ont été ultérieurement identifiés grâce à la suspicion clinique, suivie d'un test de génotypage par sonde linéaire (LPA). La tuberculose multirésistante (TB-MR) représentait 46,4 % des cas (n = 13), la monorésistance à la rifampicine 39,3 % (n = 11) et la monorésistance à l'isoniazide 14,3 % (n = 4). Deux cas de tuberculose pré-ultrarésistante (pré-TB-XDR) ont été observés chez des personnes vivant avec le VIH ; ces deux cas présentaient des résultats GeneXpert faussement négatifs. Le délai diagnostique moyen entre le test GeneXpert et le diagnostic par LPA était de 16 jours.
Conclusion: L'évaluation clinique est essentielle pour pallier les lacunes diagnostiques dues aux résultats faussement négatifs du test GeneXpert, garantissant ainsi une identification et un traitement précoces de la TB-DR.

Mots-clés: Lacunes diagnostiques, Nigéria, GeneXpert, Tuberculose

Introduction:

Drug-resistant tuberculosis (DR-TB) is drug-resistance of *Mycobacterium tuberculosis* to any of the most effective medications used in treatment regimens of TB. DR-TB can be grouped as secondary drug resistance when it results from an on-going resistance acquisition or primary drug resistance when it is from person-to-person transmission in a treatment-naive (new) patient (1). It can also be categorized as mono-drug resistance, in which there is resistance to only one drug used for treatment. Poly-drug resistance is resistance to at least two drugs used for TB treatment but not isoniazid and rifampicin. Multidrug-resistance (MDR-TB) is resistance to at least isoniazid and rifampicin, the most effective first-line TB treatment drugs. Another form of DR-TB is pre-extensively drug-resistant TB (pre-XDR TB) where *M. tuberculosis* is resistant to isoniazid, rifampicin and a fluoroquinolone. The last form of DR-TB is extensively drug-resistant TB (XDR TB) which has a resistance profile to isoniazid and rifampicin, a fluoroquinolone, and either bedaquiline or linezolid (1–3).

The initiative for eliminating TB cannot be successful until DR-TB is adequately stemmed (4). DR-TB is part of the broader framework of global antimicrobial resistance (AMR) occurrence which is estimated to accrue more morbidity and mortality in Africa (5). DR-TB is estimated to cause 13% of all AMR-attributable deaths worldwide (6). Nigeria is recently ranked 6th among 30 high-burden TB countries globally and 14th among countries with DR-TB based on estimated incidence of MDR-TB while the national MDR-TB burden is estimated to be 21,000 annually (7). Further, the prevalence of MDR-TB in Nigeria is estimated at 4.3% among new TB cases (treatment-naive) and 25% among previously treated cases (8). A pooled Southwestern region prevalence in Nigeria of MDR-TB in 2023 was about 13.8% (9) despite the pragmatic approach of combating DR-TB in Ekiti State with early identification and management of DR-TB cases.

Diagnostic and treatment gaps appear to worsen the present situation, deterring the achievement of the end TB strategy (4). Initial diagnosis of pulmonary TB (PTB) is majorly by sputum GeneXpert MTB/RIF assay (7). Although this diagnostic has an impressive predictive value, it could still present with false-negative results in some circumstances (10). This would require heightened clinical evidence to bridge the apparent diagnostic gap to achieve a good clinical outcome. We therefore identified the relevance of clinical diagnoses in supplementing diagnostic gaps of DR-TB in Ekiti State, Nigeria.

Materials and method:

A secondary data analysis was conducted using the 2024 register of the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP) in Ekiti State, Nigeria. The register included patients diagnosed with DR-TB across various local government areas within the State. Data extracted from the register included patient demographics (age, sex),

HIV status, mode of TB diagnosis (GeneXpert MTB/RIF assay, line probe assay [LPA], and/or culture), drug-resistant patterns, TB treatment category (new or previously treated), and diagnostic timelines. Only confirmed DR-TB cases recorded in the 2024 register were included. Incomplete entries or unverified diagnoses were excluded from analysis.

Ethical approval for this study was obtained from the Ekiti State Ministry of Health and Human service Ethics Committee**.** Approval number MOH/EKHREC/EA/P/139**.** No individual identifiers were collected. Dataset was stored a pass-worded computer and could only be accessed by authors for analysis.

Descriptive statistics, including frequencies and percentages were used to summarize the data. Trends in diagnostic outcomes and resistance patterns were also described. Graphical representations were generated for geographic distribution and DR-TB categories.

Results:

A total of 28 people were diagnosed with drug-resistant TB in 2024, with female to male ratio of 9 to 19 and mean age of 38±13.8 years. Only four patients (14.3%) were people living with HIV/AIDS (PLWHA) and were on anti-retroviral medications as at DR-TB diagnosis. Of these, 1 was a new case, while the other 3 were previously treated TB cases (relapse or treatment after follow-up). Furthermore, 2 of the 4 (50%) PLWHA had false-negative sputum GeneXpert MTB/RIF assay.

Sputum GeneXpert MTB/RIF assay identified 15 (53.6%) of the 28 patients as DR-TB and missed 13 (46.4%). These 13 sputum GeneXpert MTB/RIF assay-negative cases were evaluated on clinical premise with(out) radiology, which necessitated further sputum analysis using line probe assay (LPA). LPA was positive in all the 28 DR-TB cases (sputum GeneXpert MTB/RIF assay positive and negative) with highest identification as MDR-TB (13/28, 46.4%), followed by rifampicin mono-resistant TB (11/ 28, 39.3%) and isoniazid mono-resistant TB (4/28, 14.3%) (Fig 2). In addition, LPA identified the second line anti-TB drug resistance in 2 HIV-positive patients showing intermediate susceptibility to both fluoroquinolones and aminoglycosides in one and only fluoroquinolones in another, indicating pre-XDR.

For those with false-negative sputum GeneXpert MTB/RIF assay, the timeline difference between a negative sputum GeneXpert MTB/RIF assay and a positive LPA, which necessitated initiation of DR-TB medications, was averagely 16±23 days. Of the 9 (32.1%) patients who had subsequent culture of sputum for further analysis, only 4 (44.4%) were culture positive. Only one patient (3.6%) died from this pool of patients managed as DR-TB in 2024.

Fig 1 displayed the distribution of the DR-TB cases by Local Government Areas in Ekiti State, with Ado-Ekiti having the highest frequency of cases (35.7%, n=10) in the State. Fig 2 displayed the frequency of the DR-TB patients' category with drug-resistance patterns using LPA, with MDR-TB mostly seen in persons who has been previously treated for TB. Fig 3 displayed the distribution of sputum GeneXpert MTB/RIF assay results among DR-TB patients' category, with false-negative sputum GeneXpert MTB/RIF assay prevalent in previously treated TB cases (61.5%, n=8/13).

Fig 4 presented the prevalence of DR-TB in Ekiti State over the three-year period of the study, which showed an initial high rate of 5.1% (n=23/450) in 2022, followed by a decline to 2.9% (n=15/515) in 2023 and a rise to 3.5% (n=28/781) in 2024.

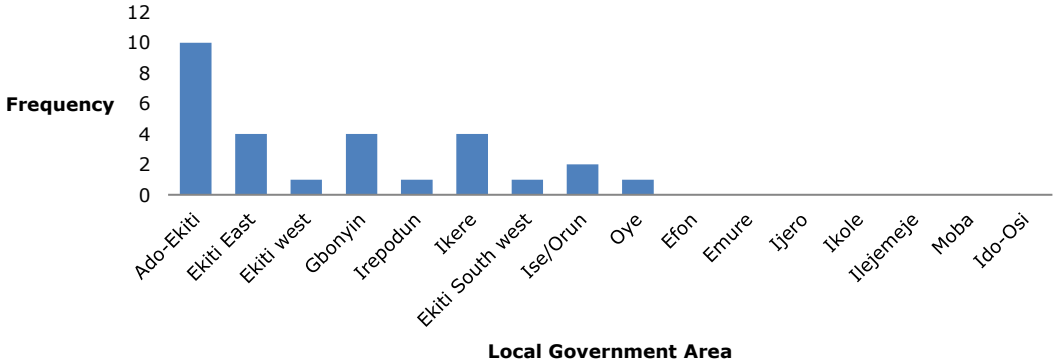


Fig 1: Distribution of drug-resistant tuberculosis (DR-TB) cases by Local Government Areas in Ekiti State, Nigeria, 2024.

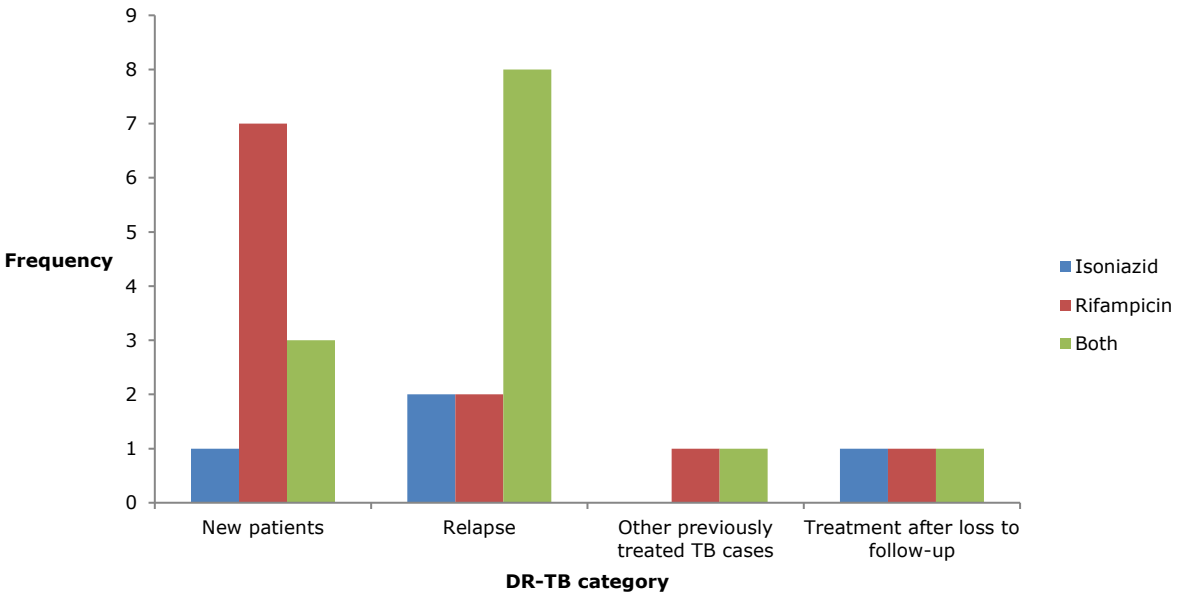


Fig 2: Distribution of drug-resistant tuberculosis (DR-TB) patients with drug-resistance patterns using LPA in Ekiti State, 2024

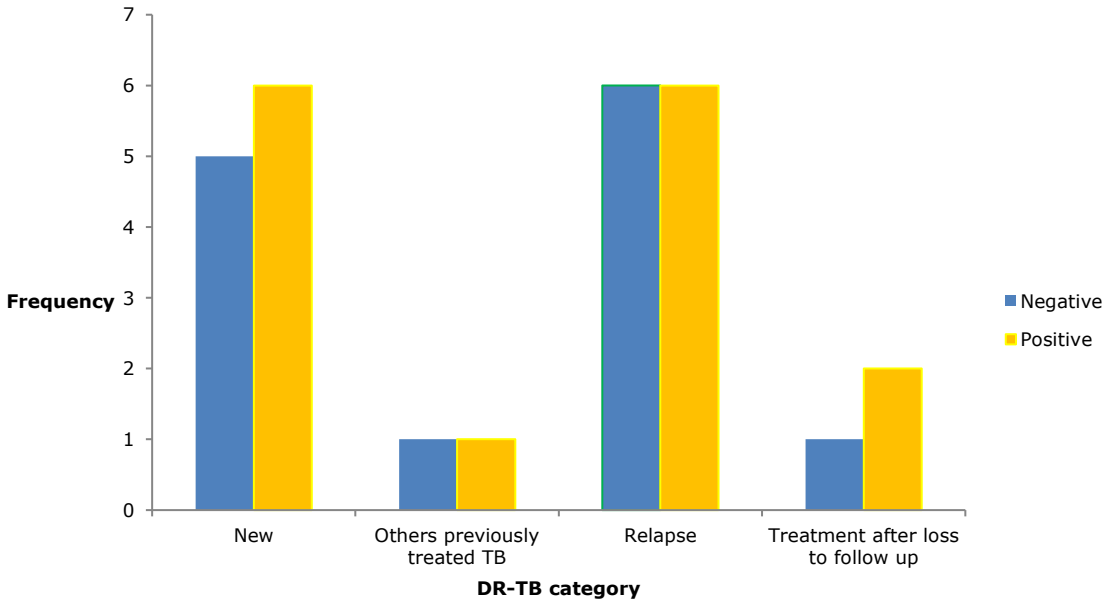


Fig 3: Distribution of sputum GeneXpert results among drug-resistant tuberculosis (DR-TB) patients' category

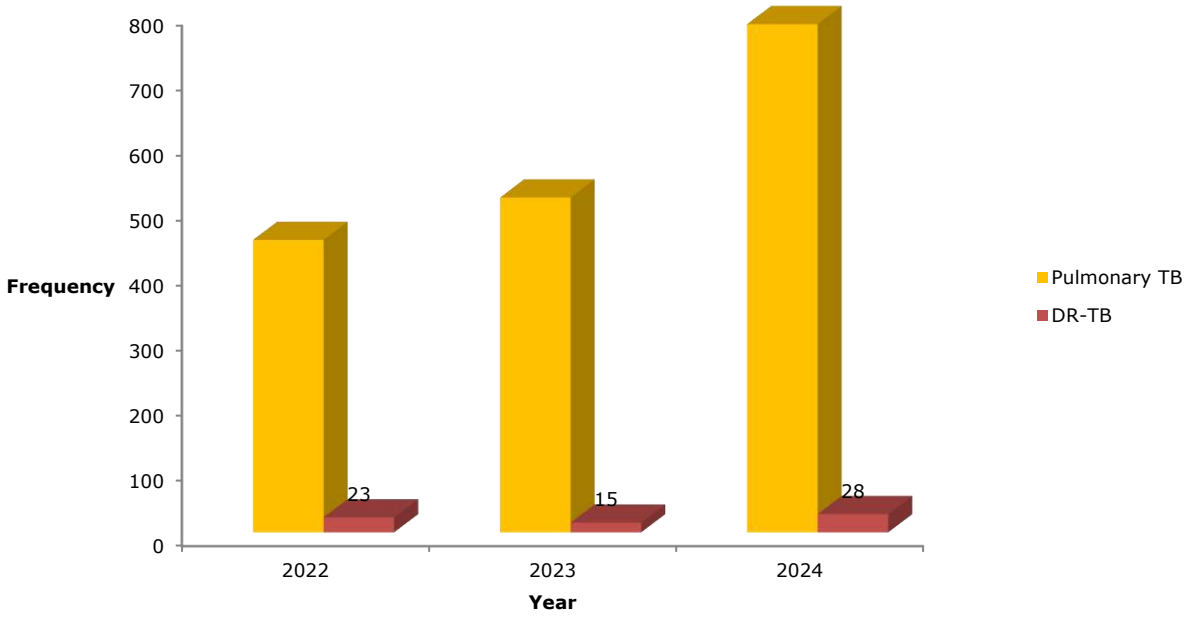


Fig 4: Frequency of pulmonary and drug-resistant tuberculosis in Ekiti State, Nigeria, over a 3-year period

Discussion:

The occurrence of DR-TB is a global challenge, and its burden is more observed in Sub-Saharan Africa due to many factors such as inadequate TB diagnostics, limited access to quality healthcare and relative inaccessibility to anti-TB medications amidst other social factors (9,10). This has contributed to ineffective drug uptake of DR-TB medication by about 40% (11). Clinical diagnosis of TB has apparently increased presumptive cases of TB where diagnostic gap exists. Further confirmation with more specific TB diagnostics such as line probe assay and or culture would be helpful in early identification and commencement of anti-TB medications. Hence, diagnostic and treatment gaps ensue when the initial TB diagnostics fail.

In our study, Ado-Ekiti had the highest number of DR-TB cases (n=10, 35.7%) in the State. Being the state capital and urban area with population density estimated about 1,100/ km^2 (12), Ado-Ekiti may be over-crowded according to the minimum density for such a landscape (13). Over-crowding is a known factor for person-to-person transmission of pulmonary TB (9). DR-TB was identified mostly in TB-relapse patients and secondarily in new patients (treatment–naive). The global proportion trend of DR-TB shows similar occurrence where DR-TB is seen in previously treated patients than in new patients (2). While the TB-relapse patient had a high rate of MDR-TB (i. e. resistance to both isoniazid and rifampicin), the new patients had rifampicin mono-resistance (RMR) as most prevalent drug-resistant pattern. MDR-TB still remains the major drug-resistant pattern observed in TB (2,14) and their occurrence in previously TB treated patients is not unexpected due to inadequate drug compliance, or drug pharmacokinetics in the individuals (1,5).

The diagnosis of RMR is usually done with LPA which was adopted by the WHO for testing DR-TB (6,15). This increasing RMR is gaining global attention, although studies are sparse on its occurrence, it has been noted to be associated with HIV and with poorer clinical outcomes (15–17). A recent study in south-western region of Nigeria identified a proportion of 6.7% among DR-TB patients (8). This rate is lower than our findings of 39.3% (11/28). This may require more extensive studies on a larger premise to further evaluate these discrepancies.

The only mortality recorded in our study was identified as RMR which concurs with poor clinical outcomes as noted by aforementioned studies. PLWHA in our study were not identified with RMR, contrary to prevalent studies (16,17) where a quarter of PLWHA were diagnosed with RMR in South Africa (17). Our negative finding from PLWHA may be due to the small sample of PLWHA in our pool of

dataset analysis. Isoniazid-resistant TB in our dataset was less than a quarter of all DR-TB patients. Global rate of this resistant pattern is about 17%, with 7.1% as new cases and about 10% in previously treated TB cases (2), which is in tandem with our study. Olabiyi et al., (8) reported a prevalence of isoniazid-resistant TB among DR-TB of about 4%. In Taiwan, the total proportion of isoniazid-resistant TB was about 1:20 persons (18). These figures are notwithstanding lower than rate obtained in our study. Therefore, Ekiti State may be harbouring more isoniazid-resistant TB than presently identified if extensively studied. Inappropriate use of isoniazid preventive therapy as TB preventive treatment among contacts of pulmonary TB or TB prophylaxis for PLWHA might be a reason for the occurrence of such resistance pattern (19).

HIV has been a duet with TB in common occurrence (10). Less than 1 in 20 of the DR-TB patients in our dataset were PLWHA. These patients were nonetheless on anti-retrovirals and were judged to be adherent on medications. Only one of PLWHA was a new case. This patient was likely to have had a lower CD4+ count at the commencement of anti-retrovirals and perhaps not placed on tuberculosis preventive therapy. Two of PLWHA had false-negative sputum GeneXpert MTB/RIF assay results and were both identified to be Pre-XDR TB on LPA. False-negative sputum GeneXpert MTB/RIF assay in PLWHA is quite common (6,10) and they are predisposed to worse forms of DR-TB due to relative immune anergy (20). From Southeast (21) to Southwestern Nigeria (22), the prevalence of pre-XDR has ranged from 7.1% to 16.7% respectively whereas our dataset just identified two (2/28, 0.07%).

The discrepancies noted with false-negative TB have been a global problem and have perpetuated diagnostic and treatment gaps (10). About half of the patients in our dataset diagnosed of DR-TB had a false-negative sputum GeneXpert MTB/RIF assay. GeneXpert MTB/RIF assay is the mainstay of diagnosis of TB in Nigeria (7) hence, thorough clinical evaluation with (out) radiologic evidence of pulmonary TB (24) can supplement for patients with false-negative sputum GeneXpert MTB/RIF assay result to further investigate the patient with either LPA with/out culture. Traditional symptoms such as chronic cough with (out) haemoptysis, weight loss, night sweats are not exclusive of pulmonary tuberculosis. Therefore, identifying this constellation of symptoms especially in patients who have previously been treated for TB or in PLWHA should heighten clinical suspicion of DR-TB as rightfully highlighted in the national guidelines (7,8).

Unfortunately, diagnostic gap in a very contagious disease such as Pulmonary TB could lead to rapid person-to-person transmission. As these patients with false-negative results could infect close contacts leading to an overt community transmission. In this study, it took an average of 16 days to make up for the diagnostic gap and commence DR-TB medication. Since one active untreated pulmonary TB patient have the capacity of infecting 15-20 persons per year (2), it can be deduced from our study that an average of one contact each of the initial missed cases could have been infected during the 16-day diagnostic gap. This is alarming based on the estimation of the reduction proportion of the end TB strategy (4) .

A three-year assessment of the prevalence of pulmonary TB with DR-TB in Ekiti from 2022 to 2024 shows an increase in total pulmonary TB in the state, however, there was an initial reduction of DR-TB from 2022 to 2023, while 2024 saw a gradual increase in prevalence compared to 2023. This might be due to the added clinical diagnosis that bridged the diagnostic gap as was seen in a study in Nigeria by Oga-Omenka et al., (25). Nigeria has been experiencing a gradual but constant rise in incidence of DR-TB (26) whereas global trend of DR-TB observed a plateau of incidence within 2020-2023 (2). This global trend could change if low-and middle-income countries increase clinical suspicion to further identify DR-TB.

Our study has some limitations. First, the small dataset limits generalizability and statistical inference. Second, there are inadequate important clinical and socioeconomic variables in the dataset, precluding multivariate analysis of risk factors for specific drug-resistant patterns. Third, reliance on programmatic data as with ours may have introduced reporting biases. Lastly, the absence of long-term treatment outcome data limits assessment of clinical effectiveness and mortality trends.

Conclusion:

Nigeria remains a high-burden country for DR-TB with diagnostic limitations potentially leading to underreporting and delayed treatment. This study highlights the importance of integrating clinical judgment supported by radiologic evaluation and confirmatory assays such as LPA to obtund diagnostic and treatment gaps especially in previously treated TB patients and PLWHA with false-negative sputum GeneXpert MTB/RIF assay. This is imp-

ortant to reduce diagnostic timeline and curb community transmission to finally enable national and global targets for DR-TB control and elimination.

Acknowledgments:

The authors wish to appreciate the Ekiti State team of National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP).

Contributions of authors:

EOI conceptualized the study; EOI, AOI, and JAO were involved in study investigation; EOI was involved in methodology; EOI and AOI were involved in formal analysis; EOI wrote the original manuscript draft: EOI, AOI, and JAO reviewed and edited the manuscript. All authors approved the manuscript submitted for publication.

Source of funding:

Authors received no external funding

Conflict of interest:

Authors declare no competing interests

Disclosure:

Part of the entire set of findings in this study have been presented in a conference as a poster presentation in World Conference on Lung Health 2025, Copenhagen, Denmark.

References:

1. Clinical Overview of Drug-Resistant Tuberculosis Disease | Tuberculosis (TB) | CDC https://www.cdc.gov/tb/hcp/clinical-overview/drug-resistant-tuberculosis-disease.html. (Accessed May 2, 2025).
2. Global tuberculosis report 2024. Geneva: World Health Organization: Licence: CC BY-NC-SA 3.0 IGO.; 2024: 69
3. Lewinsohn, D. M., Leonard, M. K., Lobue, P. A., et al. Official American Thoracic Society/Infectious Diseases Society of America / Centers for Disease Control and Prevention Clinical Practice Guidelines Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis. 2017; 64 (2): e1–e33. doi: 10.1093/cid/ciw694.
4. World Health Organization. Compendium of WHO guidelines and associated standards: ensuring optimum delivery of the cascade of care for patients with tuberculosis. World Health Organization. 2018: 48. https://apps.who.int/iris/bitstream/handle/10665/272644/9789241514101-eng.pdf?ua=1
5. O 'Neill, J. Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance. Wellcome Trust; 2016: 84. https://amr-review.org/sites/default/files/160518
6. Farhat, M., Cox, H., Ghanem, M., et al. Drug-resistant tuberculosis: a persistent global health concern. Nat Rev Microbiol. 2024 22(10): 617-635. doi: 10.1038/s41579-024-01025-1
7. National Guidelines for Clinical and Programmatic Management of Drug-Resistant Tuberculosis in Nigeria. Programme, Department of Public Health National Tuberculosis and Leprosy Control; 2020: 133
8. Kwaghe, A. V., Umeokonkwo, C. D., and Aworh, M. K. Evaluation of the national tuberculosis surveillance and response systems, 2018 to 2019: National tuberculosis, leprosy and Buruli ulcer control programme, Abuja, Nigeria. Pan Afr Med J. 2020; 35: 1–11. doi: 10.11604/pamj.2020.35.54.21493
9. Olabiyi, O. E., Okiki, P. A., Adarabioyo, M. I., et al. Distribution of multi-drug-resistant tuberculosis in Ekiti and Ondo states, Nigeria. New Microbes New Infect. 2023; 55: 101192. doi.org/10.1016/j.nmni.2023.101192
10. Velen, K., Podewils, L. J., Shah, N. S., et al. Performance of GeneXpert MTB/RIF for diagnosing tuberculosis among symptomatic household contacts of index patients in South Africa. Open Forum Infect Dis. 2021; 8 (4): doi: 10.1093/ofid/ofab025
11. Liebenberg, D., Gordhan, B. G., and Kana, B. D. Drug resistant tuberculosis: Implications for transmission, diagnosis, and disease management Front Cell Infect Microbiol. 2022; 12: 1–18. doi: 10.3389/fcimb.2022.943545
12. Nyasulu, P. S., Doumbia, C. O., Ngah, V., Togo, A. C. G., Diarra, B., and Chongwe, G. Multidrug-resistant tuberculosis: latest opinions on epidemiology, rapid diagnosis and management. Curr Opin Pulm Med. 2024; 30 (3): 217–228. doi: 10.1097/MCP. 0000000000001070
13. World Health Organization. WHO consolidated guidelines on tuberculosis. WHO Press. 2022: 98
14. Sunday, A. S. A. P., and Jibo, A. A meta-analysis of Nigeria's population census results falsification and its implications. Wukari International Studies Journal. 2024;8(3):81–91.
15. UN Habitat. United Nations Human Settlement Programme (Un-Habitat) Annual Report 2022. 2022: 31–70. https://unhabitat.org/wcr/
16. Malenfant, J. H., and Brewer, T. F. Rifampicin Mono-Resistant Tuberculosis - A Review of an Uncommon but Growing Challenge for Global Tuberculosis Control. Open Forum Infect Dis. 2021; 8 (2): 1–6. doi: 10.1093/ofid/ofab018
17. Gibson, J., Donnan, E., and Eather, G. Management of rifampicin mono-resistant tuberculosis in Queensland, Australia: a retrospective case series. Respirol Case Rep. 2018; 6 (8): 1-4. doi: 10.1002/rcr2.366
18. Salaam-Dreyer, Z., Streicher, E. M., Sirgel, F. A., et al. Rifampicin-mono resistant tuberculosis is not the same as multidrug-resistant tuberculosis: A descriptive study from Khayelitsha, South Africa. Antimicrob Agents Chemother. 2021; 65 (11). doi: 10.1128/AAC.00364-21
19. Chien, J. Y., Chen, Y. T., Wu, S. G., Lee, J. J., Wang, J. Y., and Yu, C. J. Treatment outcome of patients with isoniazid mono-resistant tuberculosis Clin Microbiol Infect. 2015; 21 (1): 59–68. doi: 10.1016/j.cmi.2014.08.008
20. Balcells, M. E., Thomas, S. L., Godfrey-Faussett, P., and Grant, A. D. Isoniazid preventive therapy and risk for resistant tuberculosis. Emerg Infect Dis. 2006; 12 (5): 744–751. doi: 10.3201/eid1205.050681

21. World Health Organization Companion handbook. WHO. 2014: 464 http://apps.who.int/iris/bitstream/10665/75146/1/9789241548441_eng.pdf
22. Nwachukwu, N. O., Ulasi, A. E., Okoronkwo, C. U., and Unegbu, V. N. Pre-extensively drug-resistant tuberculosis among pulmonary multidrug-resistant tuberculosis patients in Eastern Nigeria. Lung India. 2023; 40 (6): 492–495. doi: 10.4103/lungindia.lungindia_337_23
23. Daniel, O. J., Osman, E., Oladimeji, O., and Dairo, O. G. Pre-Extensive Drug-Resistant Tuberculosis (Pre-XDR-TB) among MDR-TB Patents in Nigeria. Glob Adv Res J Microbiol. 2013; 2 (2): 1–4.
24. Ryu, Y. J. Diagnosis of Pulmonary Tuberculosis: Recent Advances and Diagnostic Algorithms. Tuberc Respir Dis (Seoul). 2015; 78 (2): 64. doi: 10.4046/trd.2015.78.2.64
25. Oga-Omenka, C., Boffa, J., Kuye, J., Dakum, P., Menzies, D., and Zarowsky, C. Understanding the gaps in DR-TB care cascade in Nigeria: A sequential mixed-method study. J Clin Tuberc Other Mycobact Dis. 2020; 21: 100193. doi: 10.1016/j.jctube.2020.100193
26. Oladimeji, O., Atiba, B. P., Anyiam, F. E., et al. Gender and Drug-Resistant Tuberculosis in Nigeria. Trop Med Infect Dis. 2023; 8 (2): 1–11. doi: 10.3390/tropicalmed8020104

Eliya et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 277 - 285 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2489. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Impact of sputum transit time on TB isolation and culture contamination rate

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Ugwu, C., [1]Emmana, B., [1]Ishaku, P., [1]James, E., and [1,3]John, B.

[1]Zankli Research Centre, Bingham University, Karu, Nasarawa State, Nigeria
[2]Biological Sciences Department, Bingham University, Karu, Nasarawa State, Nigeria
[3]Community Medicine and Primary Health Care Department, Bingham University, Karu, Nasarawa State, Nigeria
*Correspondence to: iliya.taryuta@binghamuni.edu.ng; Tel: +2347034229746

Abstract:

Background: Tuberculosis (TB) culture is the 'gold standard' test for detecting *Mycobacterium tuberculosis* and enables comprehensive drug susceptibility testing. However, culture contamination remains a challenge in TB culture, resulting in significant loss of time and resources, and potentially leading to poor treatment outcomes due to incorrect clinical decision-making. The objective of this study is to assess the effect of sputum transit time on contamination rate and the yield of *M. tuberculosis.*
Methodology: We conducted a prospective analysis of all samples received for TB culture at a Reference Laboratory from January to December 2021. Samples were decontaminated by the standard N-Acetyl L-cysteine (NALC)/Sodium Hydroxide (NaOH) method and inoculated on Lowenstein-Jensen (LJ) solid media. The number of days from sample collection to processing was documented for all 988 valid samples received within the study period. A univariable analysis was used to explore the relationship between transit time and contamination rate. Percentages and Odds ratios with associated 95% confidence intervals were reported.
Results: Of 988 diagnoses and follow-up samples received, 508 (58.7%) were from males, while 14 (1.4%) were from children ≤ 14 years of age. Overall, 56 (5.7%) samples were contaminated, of which 17 (30.4%) were positive for AFB. A contamination rate of 4.1%, 7.1%, and 8.3% was recorded for Optimal (0-7 days), Delayed (8-14 days), and Extended delay (>15 days) samples, respectively. Compared to samples analyzed within optimal transit time, those with Delayed and Extended delay transit times had greater odds of being contaminated, with OR of 1.811 (95% CI=0.997-3.290) and 2.148 (95% CI=0.983-4.688), respectively.
Conclusion: The study revealed that TB culture contamination rate tends to increase with the number of days taken for sample movement from collection to processing. However, the association detected fell slightly short of achieving statistical significance. It is also instructive that about one in three contaminated samples were positive for AFB, which could have far-reaching implications for the clinical decision-making and management of persons being treated for various forms of TB. Investment in innovative approaches to ensure that all samples for TB culture reach the testing laboratories within the optimal period is likely to save time, resources, and lives.

Keywords: *Mycobacterium tuberculosis*, Tuberculosis-culture, contamination-rate, transit-time.

Received Jan 20, 2026; Revised Mar 20, 2026; Accepted Mar 21, 2026

Impact du temps de transit des expectorations sur l'isolement de la tuberculose et le taux de contamination des cultures

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Ugwu, C., [1]Emmana, B., [1]Ishaku, P., [1]James, E., et [1,3]John, B.

[1]Centre de Recherche Zankli, Université Bingham, Karu, État de Nasarawa, Nigéria
[2]Département des Sciences Biologiques, Université Bingham, Karu, État de Nasarawa, Nigéria
[3]Département de Médecine Communautaire et de Soins de Santé Primaires, Université Bingham, Karu, État de Nasarawa, Nigéria
*Correspondance : iliya.taryuta@binghamuni.edu.ng; Tél: +2347034229746

Résumé:

Contexte: La culture de la tuberculose (TB) est la méthode de référence pour la détection de Mycobacterium tuberculosis et permet de réaliser des tests complets de sensibilité aux médicaments. Cependant, la contamination des cultures reste un défi majeur pour la recherche de la tuberculose, entraînant des pertes importantes de temps et de ressources, et pouvant potentiellement compromettre l'efficacité du traitement en raison d'erreurs de jugement clinique. L'objectif de cette étude est d'évaluer l'influence du temps de transit des expectorations sur le taux de contamination et le rendement en mycobactéries tuberculeuses (M. tuberculosis).
Méthodologie: Nous avons mené une analyse prospective de tous les échantillons reçus pour la recherche de la tuberculose dans un laboratoire de référence, de janvier à décembre 2021. Les échantillons ont été décontaminés par la méthode standard à la N-acétyl-L-cystéine (NALC)/hydroxyde de sodium (NaOH) et ensemencés sur milieu solide de Löwenstein-Jensen (LJ). Le délai entre le prélèvement et le traitement des échantillons a été enregistré pour les 988 échantillons valides reçus pendant la période d'étude. Une analyse univariée a été utilisée pour explorer la relation entre le temps de transit et le taux de contamination. Les pourcentages et les rapports de cotes avec leurs intervalles de confiance à 95% ont été rapportés.
Résultats: Sur 988 diagnostics et échantillons de suivi reçus, 508 (58,7%) provenaient de garçons et 14 (1,4%) d'enfants de 14 ans ou moins. Au total, 56 échantillons (5,7%) étaient contaminés, dont 17 (30,4%) étaient positifs pour les BAAR. Les taux de contamination étaient respectivement de 4,1%, 7,1% et 8,3% pour les échantillons ayant bénéficié d'un délai de transit optimal (0 à 7 jours), retardé (8 à 14 jours) et prolongé (> 15 jours). Comparativement aux échantillons analysés dans les délais de transit optimaux, ceux ayant bénéficié d'un délai de transit retardé ou prolongé présentaient un risque de contamination plus élevé, avec un OR de 1,811 (IC à 95%: 0,997-3,290) et de 2,148 (IC à 95%: 0,983-4,688), respectivement.
Conclusion: L'étude a révélé que le taux de contamination des cultures de tuberculose tend à augmenter avec le nombre de jours nécessaires pour acheminer l'échantillon, du prélèvement à l'analyse. Cependant, l'association observée n'a pas tout à fait atteint le seuil de signification statistique. Il est également important de noter qu'environ un tiers des échantillons contaminés étaient positifs pour les BAAR, ce qui pourrait avoir des conséquences majeures sur la prise de décision clinique et la prise en charge des personnes traitées pour différentes formes de tuberculose. Investir dans des approches innovantes pour garantir que tous les échantillons destinés à la culture de tuberculose parviennent aux laboratoires d'analyse dans les délais optimaux permettra probablement de gagner du temps, d'économiser des ressources et de sauver des vies.

Mots-clés: *Mycobacterium tuberculosis*, culture de tuberculose, taux de contamination, délai de transit.

Introduction:

Tuberculosis (TB) continues to pose a formidable challenge to global public health, particularly in developing countries where healthcare infrastructure and resources remain limited (1). Despite significant strides in diagnosis and treatment, the disease persists with alarming prevalence (1,2). It is estimated that nearly one-third of the world's population harbors latent infection with *Mycobacterium tuberculosis* (MTB), the causative agent of TB (3). Each year, between 8 and 9 million new cases are reported, and approximately 3 million people succumb to the disease (4). These statistics underscore the urgent need for reliable diagnostic methods that can support early detection and effective treatment.

While molecular diagnostic tools have revolutionized TB detection by significantly reducing the time to diagnosis, culture remains the gold standard for confirming infection and conducting comprehensive drug susceptibility testing (DST) (5). Culture methods not only enhance diagnostic yield but also provide critical insights into the viability and resistance profile of MTB strains (6). Among the various culture techniques, the BACTEC MGIT 960 system has demonstrated superior performance, isolating mycobacteria 7–10 days faster than traditional methods such as Lowenstein-Jensen (LJ) and Middlebrook 7H10 culture media (7). In terms of both isolation rate and number of isolates, BACTEC MGIT 960 has proven to be more efficient and reliable (7).

However, the success of TB culture is influenced by several pre-analytical factors, one of the most critical being the transit time of sputum specimens from collection to processing. In remote or resource-limited settings, delays in transportation and lack of refrigeration can compromise specimen integrity, leading to increased contamination rates and reduced culture positivity (8). Interestingly, studies (9,10) have shown that sputum samples stored at −20°C for extended periods can still yield high recovery rates of MTB, even without the use of preservatives. This finding presents a cost-effective alternative for sputum storage in epidemiological and drug resistance studies, especially in low-resource environments.

A study by David et al., (11) reported a median transit time of 18 days between specimen collection and processing. Remarkably, MTB was successfully isolated from 94.4% of the samples, with only 3.7% failing to grow any mycobacteria (12). Even specimens that experienced breakthrough contamination were salvaged through a second decontamination procedure. These results suggest that single morning sputum specimens, even when refrigerated for extended periods without preservatives and transported without

refrigeration, can maintain diagnostic integrity.

Innovative transportation methods such as drone delivery, have also been explored to address logistical challenges in specimen transport. One study evaluating the impact of drone transportation on sputum quality found acceptable agreement between drone-transported samples and controls, indicating that drone delivery did not compromise specimen quality (13). The authors advocate for further research to validate this method over longer durations, potentially paving the way for its broader adoption in TB control programs.

The choice of sputum preservation method also plays a crucial role in culture outcomes. OMNIgene-Sputum (OM-S) treatment has been shown to reduce contamination rates compared to standard decontamination procedures (SDP) (14). In same-day and 5-day transit arms, contamination rates were 2% and 12% for OM-S-treated cultures, respectively, compared to 15% and 27% for SDP-treated cultures. Moreover, MTB recovery in OM-S-treated LJ cultures was significantly higher, and more in same-day and 5-day arms, respectively, than in SDP-treated cultures (15,16,17). However, OM-S-treated samples showed lower recovery rates in MGIT cultures and a 1-log reduction in viable MTB count after five days, suggesting that while OM-S improves culture quality, extended transit times may still affect bacterial viability (16,18).

Culture positivity tends to decline with increased transit time, with week 2 and week 3 specimens showing higher contamination and negativity rates compared to day-one samples (19). This trend likely reflects the diminishing viability of MTB and the proliferation of contaminating organisms over time. In a study involving 816 sputum samples preserved in cetylpyridinium chloride (CPC), 84.7% yielded MTB, while 11.9% showed no growth, 2.6% were contaminated, and 0.8% grew non-tuberculous mycobacteria (20). Notably, samples processed within two weeks showed 88.6% culture positivity, whereas those processed beyond two weeks experienced a significant decline.

OM-S has also demonstrated the ability to maintain MTB viability for up to three weeks without refrigeration, resulting in contamination rates below 0.5% (21). However, samples stored for more than two weeks exhibited delayed culture positivity, indicating that prolonged storage, even with preservatives, can affect diagnostic timelines. For sputum specimens requiring transit times of up to seven or eight days, the CPC-NaCl method has shown advantages over the standard NaOH method, reducing contamination and enhancing culture positivity on solid media within 1–2 weeks of collection (22).

Given these findings, this study seeks to evaluate the impact of sputum transit time on TB culture contamination rates. Specifically, it aims to estimate the average duration from specimen collection to arrival at the reference laboratory in north central Nigeria, and to assess how this transit time influences both contamination rates and the yield of *M. tuberculosis*. Understanding these dynamics is essential for optimizing specimen handling protocols and improving diagnostic outcomes, particularly in settings where timely transportation and refrigeration are not guaranteed.

Materials and method:

Study Setting:

The study was conducted in Zankli Research Center and health facilities located across the North Central region of Nigeria, a zone comprising both urban and rural communities with varying levels of access to tuberculosis diagnostic services. Participants were recruited from the period of 21st May – 30th October 2025 in selected primary, secondary, and tertiary health facilities where individuals presenting with symptoms suggestive of TB were undergoing routine screening. Rifampicin-resistant cases were then referred for TB culture and drug susceptibility testing at Zankli Research Center, as the zonal TB Reference laboratory. Sputum samples collected at these facilities were transported to the Zankli Research Centre, located within Bingham University, Karu, Nasarawa State, for TB culture and further laboratory analysis.

Zankli Research Centre is a specialized diagnostic and research laboratory equipped with biosafety level 2+ (BSL-2+) facilities, and it serves as a referral laboratory for TB diagnostics, including culture, drug susceptibility testing, and molecular assays. The centralization of TB culture testing at Zankli Research Centre ensured the application of standardized laboratory procedures, minimized inter-laboratory variability, and enabled accurate determination of culture outcomes. This setting allowed for the assessment of sputum transit times from peripheral health facilities to the central laboratory, which was a key variable in the study.

Ethical considerations:

Ethical approval was obtained (NHREC/21/05/2005/01501) from the Bingham University Institutional Review Board (IRB) before commencement. Eligible participants were informed about 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.

All sputum samples were collected as part of routine TB diagnostic services, with no additional invasive procedures performed for research purposes. Demographic and clinical data were obtained using a structured data collection form, and each participant was assigned a unique identification code to ensure anonymity. No personal identifiers (e.g., names, addresses, or phone numbers) appeared in any database or publication. The study adhered to ethical principles. All data were stored securely in password-protected electronic databases and locked filing cabinets for physical forms, accessible only to authorized study personnel. Participants were informed of their right to withdraw from the study at any time without any impact on their access to routine medical care.

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 effect of sputum transit time on culture contamination rates, which could inform national TB diagnostic guidelines, improve laboratory efficiency, and enhance patient outcomes. Results were disseminated in aggregate form only, with no possibility of linking findings to individual participants or facilities.

Study design:

This study utilized a prospective approach to determine the period in days from the collection of sputum samples, their arrival in the laboratory, and their processing, and to further assess the impact of the transportation period in days on the TB culture contamination rate and the yield of *M. tuberculosis* complex

Study population and sampling strategy:

The study population comprised individuals of all genders and age groups who presented to participating health facilities in north-central Nigeria with confirmed TB-positive results on the GeneXpert platform, as the baseline testing in Nigeria, and were referred for TB culture. The facilities included primary, secondary, and tertiary healthcare centers across urban, peri-urban, and rural areas.

Inclusion and exclusion criteria:

Inclusion criteria included individuals with confirmed TB-positive, rifampicin-resistant results on the GeneXpert platform, who could provide adequate sputum specimens for TB culture (at least 3mls), and willingness to provide written informed consent (or assent with parental/guardian consent for participants under 18 years). Exclusion criteria included inability or unwillingness to provide informed consent, unable to provide adequate sputum volume and TB positive but rifampicin susceptible cases.

Sample size and justification:

A total of 980 participants were targeted for enrolment based on the laboratory's annual TB culture testing capacity and the average monthly number of samples received from North Central facilities. This sample size was considered adequate to allow meaningful statistical analysis of the association between sputum transit time and culture contamination rates, assuming an estimated contamination prevalence of 13.5% from a previous study (23), a confidence level of 95%, and a margin of error of 5%.

Sampling strategy:

A consecutive sampling approach was used. All eligible patients presenting to the participating health facilities during the study period and meeting the inclusion criteria were invited to participate until the end of the recruitment period from 21st May to 30th October 2025.

Sample and data collection:

Biological sample collection:

The biological samples collected in this study were expectorated sputum specimens from participants bacteriologically confirmed with drug resistant TB on the GeneXpert platform, which were identified by trained health workers at each facility. Each participant was instructed in proper sputum production, following the National TB and Leprosy Control Programme (NTBLCP) Guidelines (24), to ensure collection of high-quality, mucoid or purulent specimens rather than saliva. For each participant, one sputum sample was collected at the time of the participant's enrollment.

The sample was collected in a sterile, leak-proof, screw-cap container (Falcon 50 mL conical tubes) containing no preservatives. The target volume per sample was 3–5 mL of sputum. After collection, sputum specimens were immediately labeled with a unique participant identification code and placed in a triple-packaging system compliant with WHO biosafety requirements (25). Samples were stored in a cooler box containing ice packs, maintaining a temperature between 2–8°C during temporary storage at the health facility and throughout transportation to the Zankli Research Centre, Karu, Nasarawa State. Transit time was recorded from sample collection to arrival at the laboratory.

Upon receipt at the BSL-2+ laboratory of Zankli Research Centre, samples were logged and processed promptly for TB culture. If immediate processing was not possible, they were stored at 4°C for a maximum of 48 hours before culture.

Data collection tools:

Demographic and clinical data were collected using a structured case report form (CRF) developed specifically for this study. The CRF captured the following variables: Participant demographics (age, sex, address), Clinical history (symptoms, HIV status, prior TB history), Sample collection details (date, time, facility), and laboratory results. The CRF was pretested with a small subset of participants (n=10) to assess clarity, relevance, and ease of use in the local context. Feedback was used to refine the wording and sequence of questions. For HIV status, data were cross-checked with facility HIV registers when available.

Data collection procedure:

Trained health workers conducted in-person interviews with consented participants in private spaces at the health facilities, using English or local languages (Hausa, Tiv, Idoma) depending on participant preference. Where necessary, a trained interpreter assisted to ensure accuracy and cultural sensitivity. Completed CRFs were reviewed daily by field supervisors for completeness and accuracy before sample dispatch.

Decontamination and culture procedure:

The N-acetyl L-cysteine (NALC)/sodium hydroxide (NaOH) standard method was used to process the sputum samples in the BSL-2+ lab (26). To prevent the loss of mycobacterial viability, NaOH was neutralized by adding phosphate buffer after 15 min. The mixture was centrifuged at 3000_g for 15 min, and the supernatant was discarded. The sediment was used for concentrated AFB smear and Solid culture inoculation on solid media. The Ziehl-Neelsen (ZN) acid-fast staining was carried out on the smear, and the AFB smears were graded as scanty (1-9 AFB/100 fields), 1+ (10-99/100 fields), 2+ (1-10 AFB/ field), or 3+ (more than 10 AFB/field) (27).

About 0.5 ml of processed sample was inoculated onto prepared L-J slants and incubated aerobically. Weekly observation for 8 weeks was made to check for growth. ZN staining was performed on the isolates of the positive cultures to detect the presence of acid-fast bacilli

BIO-LINE SD Ag MPT64 TB test:

A loop full of the culture colonies was emulsified in 100 µl sample buffer of BIO-LINE SD Ag MPT64 TB (SD Biosensor, Gyeonggbuk-do, Korea) in a cryovial, and the content was added to the well of the rapid kit cassette. Inoculated immunochromatographic cassettes were kept at room temperature for 15 min in a Class II Biosafety cabinet and examined for the presence of the control and test bands. The appearance of the band in the "C" region confirmed the validity of the test. The additional appearance of the band in the "T" region was interpreted as positive for the presence of MPT64 Ag antigen in the test culture. No band in the "C" region was interpreted as an invalid test. Standard reference strain *M. tuberculosis* complex was used as a positive control (28).

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 cleaned dataset was 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 category, HIV status, transit time category, and contamination status) were presented as frequencies and percentages. Continuous variables (e.g., transit time in days) were also analyzed. To determine the association between sputum transit time and culture contamination rate, samples were categorized into three groups: Optimal (0–7 days), Delayed (8–14 days), and Extended Delay (>15 days). Bivariate analysis was performed to estimate odds ratios (OR) with 95% confidence intervals (CI), using the Optimal transit time category as the reference group. This allowed for quantification of the relative odds of contamination for Delayed and Extended Delay samples.

Results:

Socio-demographic characteristics and HIV status of the study participants with respect to sputum culture results:

Table 1 presents the distribution of solid TB culture results, including contaminated samples, stratified by age, gender, and HIV status. Among participants aged 0–14 years, 28.6% (4/14) had culture-positive results, while 71.4% (10/14) were negative, and no contaminated samples were recorded. In contrast, participants aged 15 years and above had a lower proportion of culture positivity (16.8%, 164/974), 77.4% (754/974) were negative, and a contamination rate of 5.7% (56/974). The difference in culture positivity and contamination rate was not statistically significant with respect to age group (p= 0.3704). With respect to gender, males showed a slightly higher culture positivity rate (19.0%, 110/580) compared to females (14.2%, 58/408).

Table 1: Sociodemographic distribution of participants and HIV status with respect to solid TB culture results and contamination rate

Characteristics	Result of solid culture (%)			Total	x^2	*p* value
	Positive	Negative	Contaminated			
Age group (years)						
0-14	4 (28.6)	10 (71.4)	0	14	1.986	0.3704
> 14	164 (16.8)	754 (77.4)	56 (5.7)	974		
Gender						
Male	110 (19.0)	430 (74.1)	40 (6.9)	580	8.766	0.0125*
Female	58 (14.2)	334 (81.9)	16 (3.9)	408		
HIV status						
Positive	20 (15.7)	102 (80.3)	5 (3.9)	127	2.393	0.6639
Negative	130 (16.7)	600 (77.2)	47 (6.0)	777		
Unknown	18 (21.4)	62 (73.8)	4 (4.8)	84		

* = statistically significant at $p<0.05$

Contamination was also more frequent among males (6.9%) than females (3.9%). Culture positivity and contamination rates were significantly higher in males than females (p= 0.0125).

When stratified by HIV status, culture positivity was observed in 15.7% (20/127) of HIV-positive participants, 16.7% (130/777) of HIV-negative participants, and 21.4% (18/84) of those with unknown HIV status. Contamination rates were highest among HIV-negative participants (6.0%), followed by those with unknown status (4.8%), and lowest among HIV-positive participants (3.9%). The difference in culture positivity and contamination rates was not significantly different with respect to HIV status (p= 0.6639).

Sputum transit time in relation to solid culture positivity and culture contamination:

Table 2 shows the distribution of solid TB culture results according to the number of days between sputum sample collection and laboratory processing. Samples processed within the optimal transit time of 0–7 days had the highest proportion of culture positivity (19.3%, 100/517) and the lowest contamination rate (4.1%, 21/517). For samples processed after a delay of 8–14 days, culture positivity declined to 14.2% (50/351), while contamination rates increased to 7.1% (25/351). Similarly, samples with extended delays of more than 14 days showed a culture positivity rate of 15.0% (18 /120) and the highest contamination rate 8.3% (10/120).

Bivariate analysis, using the Optimal category as the reference, indicated that Delayed samples (8–14 days) had 1.81 times higher odds of contamination (95% CI: 0.997–3.290), while Extended delays (>14 days) were associated with 2.15 times higher odds of contamination (95% CI: 0.983–4.688). Although both categories showed a trend toward increased contamination risk with longer transit times, the associations narrowly missed conventional statistical significance (x^2=8.084, df=4, p=0.0662)

Smear microscopy results with solid culture outcomes and contamination rate:

Table 3 compares Acid-Fast Bacilli (AFB) smear microscopy results with solid TB culture outcomes, highlighting the proportion of smear-positive but culture-contaminated samples. Among AFB-negative samples, culture positivity was 7.3% (56/762), with the majority yielding negative culture results (87.5%) and a contamination rate of 5.1% (39/762).

In smear-positive categories, culture positivity increased progressively with higher smear grades. Samples graded as "scanty" on AFB microscopy had 24.2% (8/33) solid culture positivity and a 6.0% contamination rate, while those graded as 1+ showed 40.2% (33/82) culture positivity and 7.3% contamination. Samples graded 2+ demonstrated 52.0% (26/50) culture positivity and 8.0% contamination, while those with the highest smear grade (3+) yielded 75.0% (45/60) culture positivity and 8.3% contamination.

Notably, contamination was present across all smear categories, including high-grade smear positives, with 17 out of 56 contaminated samples being smear positive. The association between AFB microscopy with culture positivity and contamination rate was statistically significant (x^2=279.99, df=8, $p<0.0001$).

Table 2: Bivariate analysis of sputum transit time in relation to solid culture positivity and culture contamination

Days from sample collection to processing	Result of Solid Culture (%)			OR (95% CI)	x^2	*p* value
	Positive	Negative	Contaminated			
Optimal (0-7)	100 (19.3)	396 (76.6)	21 (4.1)	1.521 (1.089-378)	8.084	0.662
Delayed (8-14)	50 (14.2)	276 (78.6)	25 (7.1)	1.811 (0.997-3.290)		
Extended Delayed (**>**14**)**	18 (15.0)	92 (76.7)	10 (8.3)	2.148 (0.983-4.688)		
Total	**168 (17.0)**	**764 (77.3)**	**56 (5.7)**			

OR=Odd Ratio; CI=Confidence Interval; x^2 = Chi square

Table 3: Bivariate analysis of AFB smear results with solid culture positivity and contamination rate

AFB Result	Solid Culture Result (%)			x^2	*p* value
	Positive	Negative	Contaminated		
Negative	56 (7.3)	667 (87.5)	39 (5.1)	279.99	<0.0001*
Scanty	8 (24.2)	24 (69.0)	2 (6.0)		
1+	33 (40.2)	43 (52.4)	6 (7.3)		
2+	26 (52.0)	20 (40.0)	4 (8.0)		
3+	45 (75.0)	10 (16.7)	5 (8.3)		
Total	**168 (17.0)**	**764 (77.3)**	**56 (5.7)**		

x^2=Chi square 279.99, * = statistically significant at $p<0.05$

Discussion:

This study assessed the impact of sputum sample transit time on the positivity of TB culture and contamination rates among patients with drug-resistant TB in northcentral Nigeria. The key findings show that shorter transit times (0–7 days) were associated with higher culture positivity (19.3%) and lower contamination rate (4.1%), whereas delays of more than 7 days were linked to reduced positivity and increased contamination rates. Although bivariate analysis indicated increased odds of contamination with delays (OR: 1.81 for 8–14 days; OR: 2.15 for >14 days), the associations narrowly missed statistical significance (p=0.0662). Additionally, contamination was observed across all smear grades, including high-grade positives, with 17 of 56 contaminated samples being smear-positive.

Our findings reinforce existing evidence (12,13) that prolonged transit times compromise TB culture integrity. Similar studies (9, 10,13) in high-burden settings have reported that delays facilitate the overgrowth of contaminating flora, reduce MTBC viability, and diminish diagnostic yield. The trend of increasing contamination with longer delays, even without statistical significance, is operationally important, as culture loss directly affects drug susceptibility testing (DST) capacity (29). The observation that a third of contaminated samples were smear-positive echoes earlier reports (30), highlighting that even samples with high bacillary load are vulnerable to contamination if processing is delayed.

The demographic patterns showing higher culture positivity in children (28.6%) compared to adults (16.8%) may reflect sample prioritization or differences in clinical presentation. The slightly but significantly higher positivity and contamination among males may be linked to higher TB burden or occupational exposure risks in this group as reported by a study (31). In HIV-positive participants, lower positivity rates (15.7%) are consistent with the lower bacillary load seen in immunocompromised hosts, while lower contamination (3.9%) might reflect more cautious laboratory handling.

The correlation between smear grading and culture positivity observed here follows well-documented (32) trends; higher smear grades correspond with higher culture yields. However, the occurrence of contamination even in 3+ smear samples underscores that sample quality is not the only part of the equation; timely and proper handling remains crucial. These findings have operational and policy implications. Delays in sputum transport should be minimized by strengthening specimen referral networks, using dedicated transport systems, and considering innovations such as same-day courier services or molecular testing closer to the point of care. Where culture remains essential, especially for DST, the introduction of sample-preserving media could help mitigate contamination risk during extended transit.

From research perspective, further stu-

dies are needed to quantify the impact of environmental and transport conditions, as well as to evaluate cost-effective interventions to reduce delays. For policy, our findings support the integration of rapid molecular diagnostics at peripheral health facilities to reduce reliance on central laboratories for initial diagnosis.

This study had some limitations. First, as a prospective observational design, it cannot establish causality between transit time and contamination. Second, environmental factors during transport, such as temperature and storage conditions, were not systematically recorded, limiting the ability to directly link them to culture outcomes. Third, the relatively small number of pediatric samples constrained subgroup analyses. Additionally, while logistic regression trends were consistent, some associations narrowly missed statistical significance, likely due to sample size limitations in the Delayed categories.

Conclusion:

In summary, the study demonstrates that longer sputum transit times are associated with lower TB culture positivity and higher contamination rates, with potentially significant implications for TB diagnosis and treatment decision-making. Contamination in smear-positive samples represents a lost opportunity for DST and optimal patient management. Addressing transport delays through improved logistics, sample preservation methods, and expanded access to rapid diagnostics will be essential for improving TB diagnostic performance and reducing missed opportunities in care.

Acknowledgements:

The authors gratefully acknowledge the Director and all staff of Zankli Research Center for their unwavering support and for providing an enabling environment to conduct the laboratory work for this study. We extend our appreciation to the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP), the TB programme managers, the quality assurance officer, and the dedicated staff of the selected health facilities across North Central Nigeria for their collaboration and commitment throughout the study. We also thank the Institute of Human Virology Nigeria (IHVN) for their technical and operational support.

Contributions of authors:

TTE and CU conceptualized the study, developed the study protocol, and drafted the manuscript; AB and EJ finalized and proofread the manuscript; BE, TTE and PI performed the laboratory work; CU and TTE performed the data analysis. BJ reviewed the manuscript and provided overall guidance. All authors read and approved the manuscript submitted for publication.

Source of funding:

This study did not receive any external funding but received institutional support from Zankli Research Center, Bingham University, Karu, Nigeria**.**

Conflict of interest:

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Declaration:

ChatGPT 3.5 and Grammarly were utilized to brainstorm structure and phrasing during the early stages of manuscript drafting. All intellectual contributions and final content are the result of the work of the authors. 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 (TTE) upon reasonable request, following our data sharing policy.

References:

1. Suvvari, T. K. The persistent threat of tuberculosis – Why ending TB remains elusive? J Clin Tuber Other Mycobact Dis. 2025; 38: 100510. https://doi.org/10.1016/j.jctube.2025.100510
2. Mbishi, J. V., Chombo, S., Luoga, P., et al. Malaria in under-five children: prevalence and multi-factor analysis of high-risk African countries. BMC Publ Health. 2024; 24 (1):
3. Mohammadnabi, N., Shamseddin, J., Emadi, M., et al. *Mycobacterium tuberculosis*: The Mechanism of Pathogenicity, Immune Responses, and Diagnostic Challenges. J Clin Lab Anal. 2024; 38 (23):
4. Bloom, B. R., Atun, R., Cohen, T., et al. Tuberculosis. In: Holmes, K. K., Bertozzi, S., Bloom, B. R., and Jha, P. (eds). Washington DC: The International Bank for Reconstruction and Development. The World Bank. 3rd edn. 2017. https://www.ncbi.nlm.nih.gov/books/NBK525174/
5. Nguyen, T. N. A., Anton-Le Berre, V., Bañuls, A. L., and Nguyen, T. V. A. Molecular diagnosis of drug-resistant tuberculosis: A literature review. Front Microbiol. 2019; 10: 794. https://doi.org/10.3389/fmicb.2019.00794

6. Amin, M., Afrazeh, E., Seyyedian-Nikjeh, S. F., Meskini, M., Doroud, D., and Siadat, S. D. New insight in molecular detection of *Mycobacterium tuberculosis*. AMB Express. 2024; 14 (1): 74 doi: 10.1186/s13568-024-01730-3
7. Srisuwanvilai, L. O., Monkongdee, P., Podewils, L. J., et al. Performance of the BACTEC MGIT 960 compared with solid media for detection of *Mycobacterium* in Bangkok, Thailand. Diagn Microbiol Infect Dis. 2008; 61 (4): 402–407. https://doi.org/10.1016/j.diagmicrobio.2008.03.018
8. Gavi, K., and Saini, P. Optimizing HIV Testing in Infants: Enhancing Blood Sample Collection, Transport, and Healthcare Infrastructure in Nigeria 2024. https://www.researchgate.net/publication/387169957_Optimizing_HIV_Testing_in_Infants_Enhancing_Blood_Sample_Collection_Transport_and_Healthcare_Infrastructure_in_Nigeria
9. Tessema, B., Beer, J., Emmrich, F., Sack, U., and Rodloff, A. C. Rate of recovery of *Mycobacterium tuberculosis* from frozen acid-fast bacillus smear-positive sputum samples subjected to long-term storage in northwest Ethiopia. J Clin Microbiol. 2011; 49(7): 2557–2561. https://doi.org/10.1128/JCM.02403-10
10. Kwon, B. S., Park, J. S., Shin, J. A., et al. A prospective study on the long-term storage of sputum and the recovery of nontuberculous mycobacteria. Therap Adv Respir Dis. 2023; 17: 17534666231162244. doi: 10.1177/17534666231162244
11. David, A., Scott, L. E., Da Silva, P., Mayne, E., and Stevens, W. S. Storage of *Mycobacterium tuberculosis* culture isolates in Microbank™ beads at a South African laboratory. Afr J Lab Med. 2023; 12 (1): 2172. doi:10.4102/ajlm.v12i1.2172.
12. Lumb, R., Ardian, M., Waramori, G., et al. An alternative method for sputum storage and transport for *Mycobacterium tuberculosis* drug resistance surveys. Int J Tuber Lung Dis. 2006; 10 (2): 172–177. https://doi.org/10.5588/ijtld.05.0196
13. Malamule, D., Moreira, S., Madeira, C., et al. Quality Analysis of Tuberculosis Specimens Transported by Drones versus Ground Transportation. Drones. 2022; 6 (7): 155. https://doi.org/10.3390/drones6070155
14. Azam, K., Cadir, N., Madeira, C., Gillespie, S. H., and Sabiiti, W. OMNIgene. SPUTUM suppresses contaminants while maintaining *Mycobacterium tuberculosis* viability and obviates cold-chain transport. ERJ Open Res. 2018; 4 (1): 00074-2017. doi: 10.1183/23120541.00074-2017
15. Anthony, R., Cobelens, F., Gebhard, A., and van Soolingen, D. Liquid culture for *Mycobacterium tuberculosis*: Proceed, but with caution. Int J Tuber Lung Dis. 2009; 13 (9): 1051–1053. https://doi.org/10.5588/ijtld.09.0156
16. Rivera, A. B., Tupasi, T. E., Grimaldo, E. R., Cardano, R. C., and Co, V. M. Rapid and improved recovery rate of *Mycobacterium tuberculosis* in Mycobacteria Growth Indicator Tube combined with solid Löwenstein-Jensen medium. Int J Tuber Lung Dis. 1997; 1 (5): 454–459. https://doi.org/10.5588/ijtld.97.454
17. Mambuque, E., Saavedra, B., Molina-Moya, B., et al. Evaluation of OMNIgene-Sputum for preservation of sputum samples for diagnosis of *Mycobacterium tuberculosis*. Trop Med Infect Dis. 2023; 8 (7): 367. https://doi.org/10.3390/tropicalmed8070367
18. Campelo, T. A., Cardoso de Sousa, P. R., Nogueira, L., Frota, C. C., and Zuquim Antas, P. R. Revisiting the methods for detecting *Mycobacterium tuberculosis:* what has the new millennium brought thus far? Access Microbiol. 2021; 3 (7): 000245. doi: 10.1099/acmi.0.000245
19. Pagaduan, J. V., and Altawallbeh, G. Advances in TB testing. Adv Clin Chem. 2023; 115: 33–62. https://doi.org/10.1016/bs.acc.2023.02.001
20. Das, D., Dwibedi, B., and Kar, S. K. Transportation of sputum samples in cetylpyridinium chloride for drug resistance studies from remote areas of Odisha, India. J Infect Develop Ctries. 2014; 8(8): 1059–1062. https://doi.org/10.3855/jidc.4350
21. Tagliani, E., Alagna, R., Tafaj, S., Hafizi, H., and Cirillo, D. M. Evaluation of *Mycobacterium tuberculosis* viability in OMNIgene-SPUTUM reagent upon multi-day transport at ambient temperature. BMC Infect Dis. 2017; 17(1): 735. https://doi.org/10.1186/s12879-017-2838-8
22. Selvakumar, N., Gopi, P., Venkataramu, K., Datta, M., Paramasivan, C., and Prabhakar, R. Isolation of tubercle bacilli from sputum samples of patients in field studies by the cetylpyridinium chloride-sodium chloride and sodium hydroxide methods. Indian J Med Res. 1995; 102: 149–151.
23. Ajide, B., Chuku, A., and Orole, K. Prevalence of *Mycobacterium tuberculosis* complex in North central Nigeria. Nig J Microbiol. 2020; 34(2): 5325–5332.
24. Federal Republic of Nigeria. Federal Ministry of Health Department of Public Health. National Tuberculosis, Leprosy and Buruli Ulcer Control Programme National Guidelines on the Implementation of TB Loop-Isothermal Mediated Amplification (TB-LAMP) test for the Diagnosis of Tuberculosis. https://ntblcp.org.ng/content/uploads/2023/06/National-Guidelines-for-the-Implementation-of-TB-Lamp-in-Nigeria-June-2021-1.pdf
25. WHO. Guidance on regulations for the transport of infectious substances. World Health Organization 2023.https://iris.who.int/bitstream/handle/10665/376214/9789240089525-eng.pdf
26. Bradner, L., Robbe-Austerman, S., Beitz, D. C., and Stabel, J. R. Chemical decontamination with N-acetyl-L-cysteine–sodium hydroxide improves recovery of viable *Mycobacterium avium* subsp *paratuberculosis* organisms from cultured milk. J Clin Microbiol. 2013; 51 (7): 2139–2146. https://doi.org/10.1128/JCM.00644-13
27. Selvakumar, N., Rahman, F., Rajasekaran, S., Narayanan, P. R., and Frieden, T. R. Inefficiency of 0.3% Carbol Fuchsin in Ziehl-Neelsen Staining for Detecting Acid-Fast Bacilli. J Clin Microbiol. 2002; 40 (8): 3041–3403. doi: 10.1128/JCM.40.8.3041-3043.2002.
28. SD BIOSENSOR. TB LAM Ag Test Product information. 2021. https://www.sdbiosensor.com/product/product_view?product_no=227 (Accessed Aug 13, 2025).
29. Lam, E., Nateniyom, S., Whitehead, S. J., et al. Use of Drug-Susceptibility Testing for Management of Drug-Resistant Tuberculosis, Thailand, 2004–2008. Emerg Infect Dis. 2014; 400 - 408 doi: 10.3201/eid2003.130951.
30. Musisi, E., Sessolo, A., Kaswabuli, S., et al. High *Mycobacterium tuberculosis* Bacillary Loads Detected by Tuberculosis Molecular Bacterial Load Assay in Patient Stool: a Potential Alternative for Non sputum Diagnosis and Treatment Response Monitoring of Tuberculosis. Microbiol. Spectr. 2022; 10 (1): e0210021. doi: 10.1128/spectrum.02100-21
31. Hargreaves, J. R., Boccia, D., Evans, C. A., Adato, M., Petticrew, M., and Porter, J. D. H. The social determinants of tuberculosis: From evidence to action. Am J Publ Health. 2011; 101 (4): 654–662. https://doi.org/10.2105/AJPH.2010.199505
32. Kassa, G. M., Merid, M. W., Muluneh, A. G., and Fentie, D. T. Sputum smear grading and associated factors among bacteriologically confirmed pulmonary drug-resistant tuberculosis patients in Ethiopia. BMC Infect Dis. 2021; 21(1): 238 doi: 10.1186/s12879-021-05933-y.

Zumbi et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 286 - 296 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2491. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Efflux and *mecA/mecC*-mediated-resistance in multi-drug-resistant *Staphylococcus aureus* clinical isolates from selected Ghanaian primary and tertiary hospitals

[1]Zumbi, C. N., [2]Odoi, H., *[1]Boamah, V. E., [1]Boakye, Y. D., and [1]Agyare, C.

[1]Pharmaceutical Microbiology Section, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Department of Pharmaceutical Microbiology, School of Pharmacy, University of Health and Allied Sciences, Ho, Ghana
*Correspondence to: etsiapa@yahoo.com; veboamah.pharm@knust.edu.gh;

Abstract:

Background: Globally, methicillin-resistant *Staphylococcus aureus* (MRSA) infections are associated with increased morbidity and mortality amongst patients; however, reports on MRSA infections in Ghana are mostly from tertiary healthcare facilities. This study determined the prevalence of methicillin and multiple efflux resistance mechanisms in *S. aureus* isolates from five selected primary and tertiary healthcare facilities in Ghana.
Methodology: Different clinical samples (nasal, urine and wound) were collected from patients in four primary and one tertiary hospitals in Ghana. *Staphylococcus aureus* was isolated on mannitol salt agar and preliminarily identified using conventional biochemical tests and confirmed by PCR amplification of *Spa1113* gene and Matrix Assisted Laser Desorption Ionisation–Time of Flight Mass Spectrometry (MALDI-TOF). Antibiotic susceptibility test for each isolate to selected antibiotics was determined by the Kirby-Bauer disk diffusion method, and multi-drug resistance (MDR) was defined as resistance to three or more antibiotic classes. The *mecA and mecC* genes were detected by polymerase chain reaction (PCR). Multiple efflux-pump activity of multidrug resistant isolates was assessed against ampicillin and ciprofloxacin by microbroth dilution assay with reserpine, an efflux pump inhibitor.
Results: From a total of 626 clinical samples, *S. aureus* was confirmed in 68 (10.9%). MDR was detected in 46 (67.6%) of the 68 isolates, and 28 (41.2%) were phenotypic MRSA, with *mecA* gene confirmed in 9 (32.1%) of the MRSA isolates while *mecC* gene was absent in all the isolates. MRSA isolates were more susceptible to trimethoprim-sulfamethoxazole (50.0%) compared to other selected antibiotics. Multiple efflux-pump activity was detected as contributing to resistance in 6 of 25 (24.0%) *S. aureus* isolates.
Conclusion: The high prevalence of MRSA in clinical samples mediated by *mecA* gene and possible efflux pump activity call for re-evaluation of treatment protocols of *S. aureus* infections at both primary and tertiary healthcare facilities in Ghana.

Key words: MRSA, *mecA* gene, *mecC* gene, *Staphylococcus aureus*, efflux-pump

Received Oct 23, 2025; Revised Feb 26, 2026; Accepted Mar 4, 2026

Efflux et résistance médiée par *mecA/mecC* chez des isolats cliniques de *Staphylococcus aureus* multirésistants provenant d'hôpitaux Ghanéens de soins primaires et tertiaires sélectionnés

[1]Zumbi, C. N., [2]Odoi, H., *[1]Boamah, V. E., [1]Boakye, Y. D., et [1]Agyare, C.

[1]Section de Microbiologie Pharmaceutique, Département de Pharmacie, Faculté de Pharmacie et des Sciences Pharmaceutiques, Université des Sciences et Technologies Kwame Nkrumah, Kumasi, Ghana
[2]Département de Microbiologie Pharmaceutique, École de Pharmacie, Université des Sciences de la Santé et des Sciences Connexes, Ho, Ghana
*Correspondance: etsiapa@yahoo.com; veboamah.pharm@knust.edu.gh

Résumé:

Contexte: À l'échelle mondiale, les infections à *Staphylococcus aureus* résistant à la méthicilline (SARM) sont associées à une augmentation de la morbidité et de la mortalité chez les patients. Cependant, au Ghana, les données relatives aux infections à SARM proviennent principalement d'établissements de soins tertiaires. Cette étude a déterminé la prévalence de la résistance à la méthicilline et des mécanismes de résistance à de multiples antibiotiques chez des isolats de S. aureus provenant de cinq établissements de soins primaires et tertiaires sélectionnés au Ghana.
Méthodologie: Différents prélèvements cliniques (nasaux, urinaires et de plaies) ont été collectés auprès de patients dans quatre hôpitaux primaires et un hôpital tertiaire au Ghana. *Staphylococcus aureus* a été isolé sur gélose au mannitol et au sel et identifié préliminairement par des tests biochimiques conventionnels, puis confirmé par amplification PCR du gène *Spa1113* et par spectrométrie de masse MALDI-TOF. La sensibilité aux antibiotiques de chaque isolat a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer. La multirésistance (MDR) a été définie comme une résistance à trois classes d'antibiotiques ou plus. Les gènes *mecA* et *mecC* ont été détectés par réaction en chaîne par polymérase (PCR). L'activité des pompes d'efflux des isolats multirésistants a été évaluée vis-à-vis de l'ampicilline et de la ciprofloxacine par un test de microdilution en bouillon avec la réserpine, un inhibiteur des pompes d'efflux.
Résultats: Sur un total de 626 échantillons cliniques, Staphylococcus aureus a été confirmé dans 68 cas (10,9%). Une MDR a été détectée dans 46 de ces 68 isolats (67,6%), et 28 d'entre eux (41,2%) présentaient un phénotype de SARM. Le gène mecA a été confirmé dans 9 de ces isolats de SARM (32,1%), tandis que le gène mecC était absent dans tous les isolats. Les souches de SARM étaient plus sensibles au triméthoprime-sulfaméthoxazole (50,0%) qu'aux autres antibiotiques testés. Une activité de pompes d'efflux multiples a été détectée, contribuant à la résistance chez 6 des 25 souches de S. aureus (24,0%).
Conclusion: La forte prévalence du SARM dans les échantillons cliniques, liée au gène mecA et à une possible activité de pompes d'efflux, justifie une réévaluation des protocoles de traitement des infections à S. aureus dans les établissements de soins primaires et spécialisés du Ghana.

Mots-clés: SARM, gène *mecA*, gène *mecC*, *Staphylococcus aureus*, pompe d'efflux

Introduction:

Bacterial infections are a major cause of morbidity and mortality worldwide. For several decades, antibiotics have increased the survival rate of patients. However, emergence of resistant bacteria has become a significant problem within the health sector (1). *Staphylococcus aureus* infection is reported as a menace in hospitals in most parts of the world (2). In Ghana, it has been reported as one of the most occurring MDR pathogen amongst patients reporting to regional and teaching hospitals for clinical care (3). The anterior nares and external skin surfaces of humans are frequently colonized by staphylococci causing skin, soft tissue and invasive infections (4).

The occurrence and spread of methicillin resistant *Staphylococcus aureus* (MRSA), a strain resistant to methicillin and/or oxacillin (5) in communities and health facilities is particularly worrisome (1). Synthesis of altered penicillin-binding protein (PBP) in MRSA lowers binding affinity to β-lactam antibiotics (6). Until the discovery of *mecC* gene, detection of *mecA* was the 'gold standard' in identification of MRSA (7). Although the sequence of *mec*C gene is about 70% identical to *mecA*, the strain could be missed during phenotypic testing because it contains a type XI staphylococcal-cassette-chromosome with genetic makeup similar to oxacillin-susceptible MRSA (8), hence its detection requires genetic/molecular testings.

Studies have also reported MRSA genotypes that possess neither *mecA* nor *mecC* genes suggesting involvement of other mechanisms, including mutation in the PBP genes, hyper production of β-lactamases and multidrug efflux pump activity, in resistance to oxacillin and MRSA expression (9). In Ghana, very few studies have reported prevalence of MRSA and possible mechanisms of their resistance. Continuous monitoring of prevalence and antibiograms of MRSA in healthcare facilities is essential for effective management and infections control. This study aimed to determine the prevalence and susceptibility patterns of *S. aureus* isolates and identify possible mechanisms responsible for the resistance among the isolates in selected healthcare facilities in Ghana.

Materials and method:

Study sites:

The study sites were four primary hospitals and a tertiary hospital in Ghana; Kwame Nkrumah University of Science and Technology (KNUST) Hospital, Manhyia Government Hospital, Suntreso Government Hospital, Agogo Presbyterian Hospital, and Komfo Anokye Teaching Hospital.

Ethical consideration:

The study was approved by the Committee on Human Research, Publication, and Ethics (CHRPE) of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, with approval number CHRPE/AP/354/17.

Permissions for the study were granted by the authorities of the selected hospitals, and informed consent of selected participants and their caregivers were obtained. The study was performed in accordance with the Declaration of Helsinki (10).

Sample collection and identification:

Clinical samples (333 urine, 119 wound and 174 nasal swabs) were collected from a total of 626 patients in the five primary and tertiary hospitals in Ghana, who were selected by systematic random sampling from patients with suspected cases of infection for which microbiological assessment had been requested. The samples were inoculated into 10 mL nutrient broth and incubated at 37°C for 24 hours. A loopful was streaked on mannitol salt agar (MSA) and incubated at 37°C.

Golden-yellow colonies from MSA plates were further sub-cultured on nutrient agar and incubated at 37°C, followed by Gram staining and conventional biochemical tests on the colonies, including haemolysis on blood agar, catalase and coagulase tests, for presumptive identification of *S. aureus*, with *S. aureus* ATCC 25923 used as positive control (11). Preliminary confirmation of the *S. aureus* isolates was performed using Matrix Assisted Laser Desorption Ionisation–Time of Flight (MALDI-TOF) mass spectrometry.

Molecular confirmation of *S. aureus* and detection of *mecA* and *mecC* genes:

Isolates were further confirmed by PCR amplification of *Spa1113* gene (F: 5'-TAA AGA CGA TCC TTC GGT GAGC-3', R: 5'-CAG CAG TAG TGC CGT TTG CTT-3). A simplex PCR reaction was carried out in thermal cycler (Gene Amp, ThermoFisher Scientific, Waltham, MA, USA USA), with PCR reaction in a final volume of 25µL containing 2µL of DNA template, 12.5µL of OneTaq master mix, 2µL (0.8µM) each of forward and reverse primers and 6.5µL of nuclease-free water. The PCR consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 10 min. Amplicon size ranged from 180 to 600 bp.

PCR amplifications of *mecA* and *mecC* genes were performed following the procedure described by Boamah et al., (13), using the primers *mec*A (F: 5`-ACG GTA ACA TTG ATC GCA ACG-3`, R: 5`-GGC CAA TTC CAC ATT GTT TCG-3') and *mec*C (F: 5`-GAA AAA AAG GCT TAG AAC GCC TC-3', R: 5`-GAA GAT CTT TTC CGT TTT CAG C-3'). The PCR reaction was carried out in a final volume of 25µL containing 2µL DNA template, 12.5µL OneTaq master mix, 2µL (0.8 µM) of each primer and 6.5µL nuclease-free water. The PCR reaction consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 59°C and 55°C for 1 min for *mecA* and *mecC* genes, respectively and extension at 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products for were separated using a 1.5% $^{w}/_{v}$ agarose gel at 100 V and visualized under UV light. Amplicon sizes were detected at 176 and 138 bp, respectively (12).

Antibiotic sensitivity testing:

Antibiotic susceptibility of the *S. aureus* isolates was determined following the guidelines of Clinical and Laboratory Standards Institute (CLSI). The inoculum of *S. aureus* isolate was prepared by suspending 5 to 7 well-isolated colonies in sterile normal saline and standardized to the density of 0.5 McFarland standard. Eight antibiotic disks (Oxoid Ltd, Basingstoke, UK) in eight antibiotic classes including erythromycin (ERY-15µg), oxacillin (OXA-1µg), ciprofloxacin (CIP-5µg), chloramphenicol (CHL-30µg), trimethoprim-sulfamethoxazole (SXT-25µg), tetracycline (TET-30µg), gentamicin (CN-10µg), and cefoxitin (FOX-100µg), were placed on the surface of inoculated Mueller-Hinton agar plates and incubated at 37°C for 24 hours. Zones of growth inhibition were measured, and breakpoints for sensitive or resistant estimated according to CLSI guideline (13).

Detection of multiple efflux pump activity by microdilution assay:

The efflux pump activity of 25 MDR *S. aureus* isolates was assessed using the efflux pump inhibition assay utilising the microdilution method. The MICs of ampicillin and ciprofloxacin were determined in the presence and absence of reserpine (an efflux pump inhibitor) using 96-well microtiter plates. The inoculum was prepared by suspending 5 to 7 well-isolated colonies in sterile normal saline to the density of 0.5 McFarland standard. Using a dilution factor of 2, different concentrations of the antibiotics were prepared from 0.8 to 12.8µg/mL. One hundred microliters (100µL) of double strength nutrient broth were dispensed into 5 wells. This was followed by the addition of appropriate volumes of ciprofloxacin for a given concentration (500 µg/mL) which was serially diluted by 2-fold into each well, followed by the introduction of 20 µL of the inoculum. Sterile water was then added to make a final volume of 200 µL.

The plates were incubated for 24 hours at 37°C after which 20µL of 1.25mg/mL 3-(4,5

dimethylthiazol-2-yl)-2, 5 -diphenyltetrazolium bromide (MTT) was added to the wells. Wells that showed purple colour after 30 min incubation at 37°C indicated bacteria growth and those that remained yellowish indicated inhibitory activity. The MICs of ampicillin and ciprofloxacin were re-determined in the presence of 50µg/mL concentration of the plant alkaloid reserpine (reserpine stock solution was prepared to 500µg/mL) (14,15). A two-fold reduction in the MICs of ampicillin and ciprofloxacin for the MDR isolates indicates presence of multiple efflux pump activity.

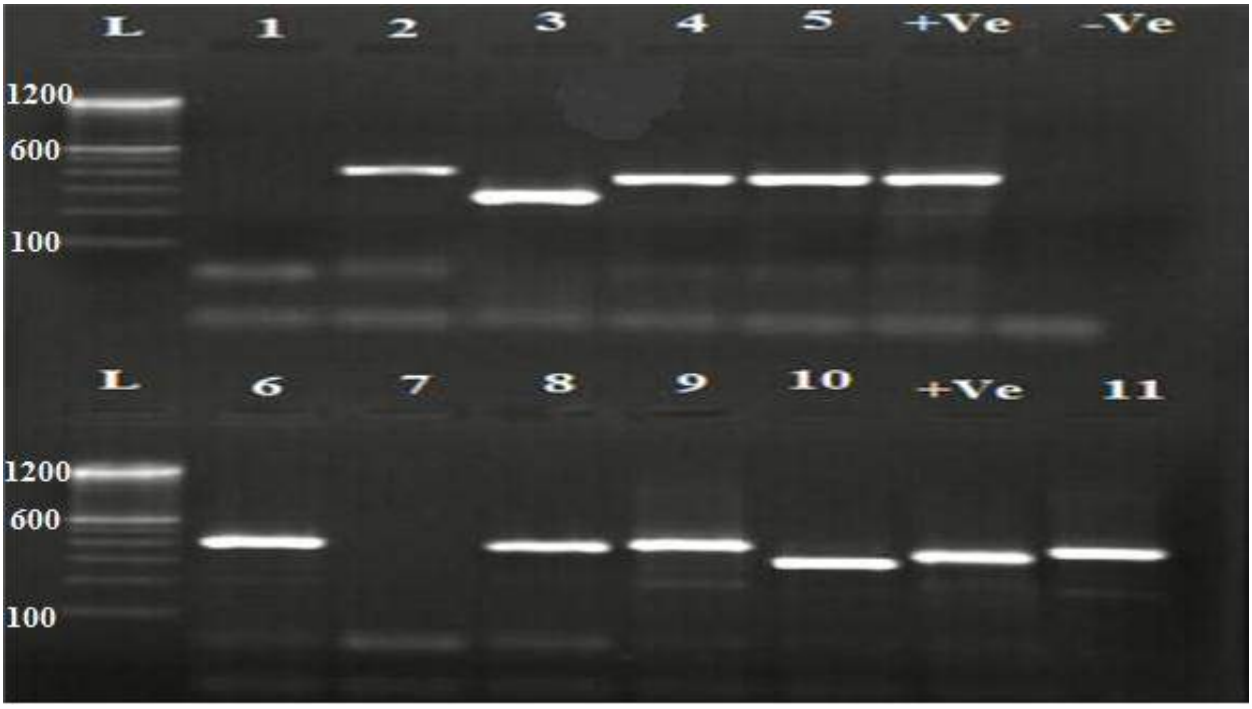
Statistical analysis:

The data generated was extracted into an MS-office Excel software Version 1908 (Microsoft Corporation Copyright 2016). Data was later analyzed using Statistical Package for the Social Sciences Version 22 (SPSS, SPSS Inc., Chicago, IL, USA) Susceptibility data were compared by using Chi-square analysis with GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). A level of significance (*p* value) less than 0.05 was considered statistically significant.

Results:

Distribution of *S. aureus* and MDR isolates in the hospitals:

Based on phenotypic growth characteristics on culture media, biochemical properties, *Spa* gene detection (Fig 1) and MALDI-TOF analysis, a total of 68 (10.9%) *S. aureus* isolates were confirmed. *S. aureus* was more frequently isolated from urine (14.7%, n=49/333) than from nasal (10.3%, n=18/174) and wound swabs (0.8%, n=1/119) (x^2=17.496, p=0.0002) (Table 1). *S. aureus* was also more frequently isolated from Agogo Presbyterian Hospital (15.3%, n=25/163) than KNUST (14.7%, n=16/109), KATH (12.0%, n=12/184), Suntreso (5.7%, n=3/53) and Manhyia (1.7%, n=2/117) (x^2= 16.843, p=0.0021) (Table 1).



L: DNA ladder; +ve: *S. aureus* 25923, -ve: RNase free water, number 1 to 11: representative *S. aureus* isolates

Fig 1: Gel image showing a 180-600 bp PCR amplicons of *Spa* gene in *Staphylococcus aureus* isolates

Table 1: Distribution of multi-drug and methicillin resistant *Staphylococcus aureus* isolates in clinical samples from primary and tertiary care hospitals in Ghana

Sources/Samples	**Total (n=626)**		**KATH (n=184)**		**Agogo (n=163)**		**KNUST (n=109)**		**Suntreso (n=53)**		**Manhyia (n=117)**	
Total *S. aureus* isolates	68 (10.9)	%	22 (12.0)	%	25 (15.3)	%	16 (14.7)	%	3 (5.7)	%	2 (1.7)	
MDR *S. aureus*	46	67.6	14	63.6	17	68.0	13	84.0	1	33.3	1	50.0
MRSA	28	41.2	7	31.8	12	48.0	9	56.3	0	0	0	0
Urine samples	**333**		**63**		**125**		**0**		**47**		**98**	
S. aureus isolates	49 (14.7)		19 (30.2)		25 (20.0)		0		3 (6.4)		2 (2.0)	
MDR *S. aureus*	31	63.2	12	63.1	17	68.0	0	0	1	33.3	1	50.0
MRSA	18	36.7	6	31.5	12	48.0	0	0	0		0	0
Nasal swab samples	**174**		**66**		**22**		**86**		**0**		**0**	
S. aureus isolates	18 (10.3)		3 (4.5)		0		15 (17.4)		0		0	
MDR *S. aureus*	15	83.3	2	66.6	0		13	86.6	0	0	0	0
MRSA	10	55.6	1	33.3	0		9	60.0	0	0	0	0
Wound swab samples	**119**		**55**		**16**		**23**		**6**		**19**	
S. aureus isolates	1 (0.8)		0		0		1 (4.3)		0		0	0

KATH: Komfo Anokye Teaching Hospital; Agogo: Agogo Presbyterian Hospital; KNUST: Kwame Nkrumah University of Science and Technology Hospital; Suntreso Government Hospital; Manhyia Government Hospital. n; number of samples.

Table 2: Susceptibility and resistance profiles of *Staphylococcus aureus* isolates from primary and tertiary healthcare facilities in Ghana

Isolate ID	**Source**	**ERY-15**	**OXA -1**	**CIP-15**	**CHL -30**	**SXT -25**	**TE-30**	**CN-10**	**FOX-30**	**MDR**
N106	Nasal	R	R	S	S	S	S	S	R	MDR
N112	Nasal	S	S	S	S	S	R	S	S	-
N114	Nasal	R	R	S	R	R	R	R	R	MDR
N116	Nasal	R	R	R	S	S	S	S	R	MDR
N119	Nasal	R	R	S	R	R	R	S	R	MDR
N126	Nasal	R	S	R	R	S	I	S	S	MDR
N134	Nasal	S	S	S	S	S	S	S	S	-
N138	Nasal	R	R	R	S	S	R	R	R	MDR
N139	Nasal	R	R	S	S	S	I	R	S	MDR
N141	Nasal	R	R	R	R	S	R	R	R	MDR
N145	Nasal	R	R	R	R	R	R	R	R	MDR
N149	Nasal	S	R	R	S	S	R	S	S	MDR
N153	Nasal	R	R	R	R	S	R	R	R	MDR
N169	Nasal	S	S	S	S	S	S	S	S	-
N175	Nasal	R	R	R	S	R	R	R	R	MDR
N34	Nasal	R	S	S	S	S	S	S	S	
N42	Nasal	S	S	R	S	S	R	S	S	
N47	Nasal	S	R	S	R	S	R	S	R	MDR
S02	Urine	I	R	S	S	R	S	S	S	-
S03	Urine	S	S	R	S	S	R	S	S	-
S28	Urine	S	R	S	R	S	R	S	R	MDR
S29	Urine	R	S	S	R	S	R	R	S	MDR
S30	Urine	R	S	R	R	S	R	S	S	MDR
S32	Urine	R	R	R	S	R	R	R	R	MDR
S40	Urine	S	S	S	R	S	R	S	S	
S42	Urine	R	R	R	R	R	R	R	R	MDR
S44	Urine	R	S	S	R	S	S	S	S	MDR
S48	Urine	S	R	S	R	S	R	S	R	MDR

S49	Urine	S	S	S	S	S	S	S	S	-
S50	Urine	S	R	S	R	R	R	R	R	MDR
S54	Urine	R	S	S	R	S	R	S	S	MDR
S55	Urine	R	S	S	S	S	R	S	S	MDR
S56	Urine	S	R	S	R	R	R	R	R	MDR
S59	Urine	S	S	S	R	S	I	S	S	-
S60	Urine	S	S	S	S	S	S	S	S	-
S79	Urine	S	S	S	S	S	S	S	S	-
Sa01	Urine	S	R	S	R	S	S	S	S	MDR
Sa03	Urine	S	S	S	S	S	S	S	S	-
Sa25	Urine	S	S	S	S	S	S	S	S	-
Sa29	Urine	S	S	S	R	S	R	S	S	-
Sa30	Urine	S	S	S	R	S	R	S	S	MDR
Sa35	Urine	S	S	S	R	S	R	S	S	MDR
Sa36	Urine	S	R	R	R	R	R	R	R	MDR
Sa40	Urine	S	R	S	R	S	R	S	S	MDR
Sa41	Urine	S	R	S	R	S	R	S	R	MDR
Sa43	Urine	R	S	S	R	S	S	S	S	MDR
Sa44	Urine	R	S	S	R	S	S	S	S	MDR
Sa48	Urine	R	R	R	R	R	R	R	R	MDR
Sa49	Urine	S	R	R	R	S	R	R	R	MDR
Sa54	Urine	R	R	S	R	R	R	S	S	MDR
Sa55	Urine	S	S	S	S	S	R	S	S	-
Sa77	Urine	S	S	S	R	S	S	S	S	-
Sa79	Urine	S	S	S	S	S	S	S	S	-
U01	Urine	R	R	R	R	R	R	R	R	MDR
U02	Urine	R	R	R	R	R	R	R	R	MDR
U09	Urine	S	R	S	S	S	R	S	S	-
U21	Urine	R	R	S	R	R	R	R	R	MDR
U24	Urine	S	S	S	S	S	R	S	S	-
U26	Urine	S	S	S	R	S	R	S	S	-
U29	Urine	R	R	R	R	S	R	R	R	MDR
U38	Urine	S	R	S	S	S	S	S	S	-
U42	Urine	R	R	R	S	S	I	R	R	MDR
U45	Urine	R	R	S	S	S	I	R	R	MDR
U49	Urine	R	R	S	S	S	R	R	R	MDR
U50	Urine	R	R	R	S	S	R	R	S	MDR
U52	Urine	S	R	S	R	S	S	S	R	MDR
U59	Urine	S	R	S	S	S	R	S	S	-
W119	Wound	S	S	S	R	S	S	S	S	-
Number of resistant isolates (%)		31 (45.5)	38 (55.8)	21 (30.9)	38 (55.8)	15 (22.1)	43 (63.2)	23 (33.8)	28 (41.2)	46 (67.6)

ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; CHL-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamicin 10ug; FOX-30: Cefoxitin-30

Antibiogram profile of MSSA and MRSA isolates from the healthcare facilities:

Of the 68 *S. aureus* isolates confirmed, 28 (41.2%) were MRSA while 40 (58.8%) were MSSA. The MRSA isolates were more prevalent in the samples from KNUST Teaching hospital (56.3%, n=9/16) compared to the others (x^2= 6.277, p=0.1794) and they were more prevalent in nasal samples (55.6%, n=10/18) compared to urine samples (36.7%, n=18/49) (OR= 0.4645, 95% CI = 0.1552 - 1.391, p = 0.2635) (Table 1). However, the MRSA prevalence differences between the hospitals and the samples were not statistically significant.

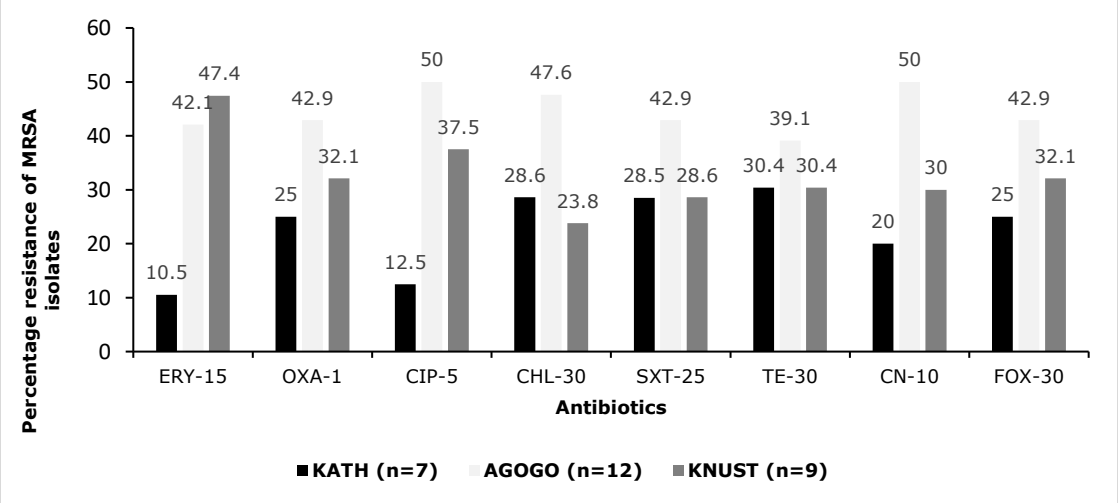
The susceptibility rates of the *S. aureus* to antibiotics tested were in the range of 28 to 84% while resistance ranged from 16 to 72% (Table 2). Most of the *S. aureus* isolates were resistant to tetracycline (63.0%, n=43/68) while the isolates showed least resistant to trimethoprim-sulfamethoxazole (22.0%, n= 15/68). Forty-six (67.6%) of the *S. aureus* isolates were multi-drug resistant (Table 2).

The MRSA isolates were resistant to all selected antibiotics, ranging between 60 to 100.0%, but were susceptible to trimethoprim-sulfamethoxazole (50.0%, n=14/28) (Fig 2a). MRSA isolates from KATH showed relatively low resistance to the different antibiotics compared to those from Agogo and KNUST hospitals (Fig 2a). Six (21.4%) of the MRSA isolates were resistant to all the eight antibiotics tested (Table 3). Erythromycin and ciprofloxacin showed relatively higher activity against the MRSA isolates from KATH while trimethoprim-sulfamethoxazole was comparatively more active against isolates from KNUST and Agogo (Fig 2a). Compared to the methicillin sensitive *S. aureus*, MRSA isolates exhibited higher resistance rates to all the antibiotics tested (Fig 2b).

Table 3: Antibiotic resistance profiles of MRSA isolates from the five selected primary and tertiary care hospitals in Ghana

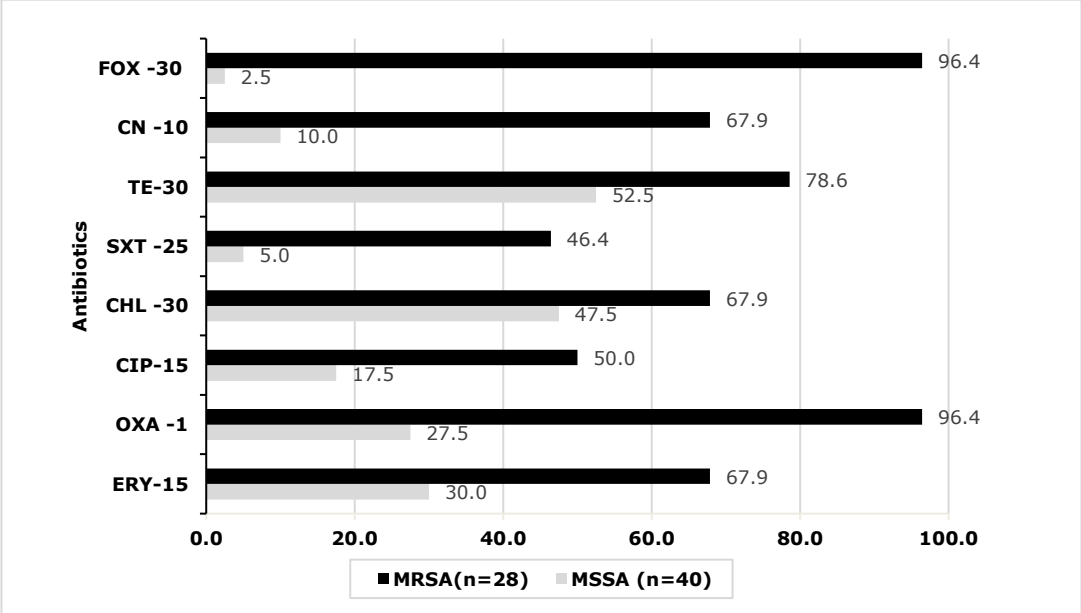
Resistance pattern	Clinical MRSA isolates (ID)+	Number of MRSA (%)
ERY-OXA-FOX	N106	1 (3.6)
OXA-CHL-FOX	U52+	1 (3.6)
ERY-OXA-CIP-FOX	N116	1 (3.6)
ERY-OXA-CN-FOX	U45	1 (3.6)
OXA-CHL-TET-FOX	S28+, N47, Sa41, S48	4 (14.3)
ERY-OXA-CIP-CN-FOX	U42	1 (3.6)
ERY-OXA-TET-CN-FOX	U49	1 (3.6)
ERY-OXA-CHL-SXT-TET-FOX	N119+	1 (3.6)
OXA-CIP-CHL-TET-CN-FOX	S49	1 (3.6)
OXA-CHL-SXT-TET-CN-FOX	S50+, S56	2 (7.1)
OXA-CIP-CHL-SXT-TET-CN-FOX	Sa36	1 (3.6)
ERY-OXA-CHL-SXT-TET-CN-FOX	U21, N114	2 (7.1)
ERY-OXA-CIP-CHL-TET-CN-FOX	U29, N141+	2 (7.1)
ERY-OXA-CIP-SXT-TET-CN-FOX	S32, N138, N175	3 (10.7)
ERY-OXA-CIP-CHL-SXT-TET-CN-FOX	Sa48+, N153+, N145, U02, S42+, U01	6 (21.4)
Total		**28 (100.0)**

ERY: Erythromycin 15 µg; OXA: Oxacillin 1 µg; CIP: Ciprofloxacin 5 µg; CHL: Chloramphenicol 30 µg; SXT: Trimethoprim-sulphamethoxazole 23.75ug; TET: Tetracycline 30ug; CN: Gentamycin 10ug; FOX: Cefoxitin-30ug; + presence of *mecA* gene.



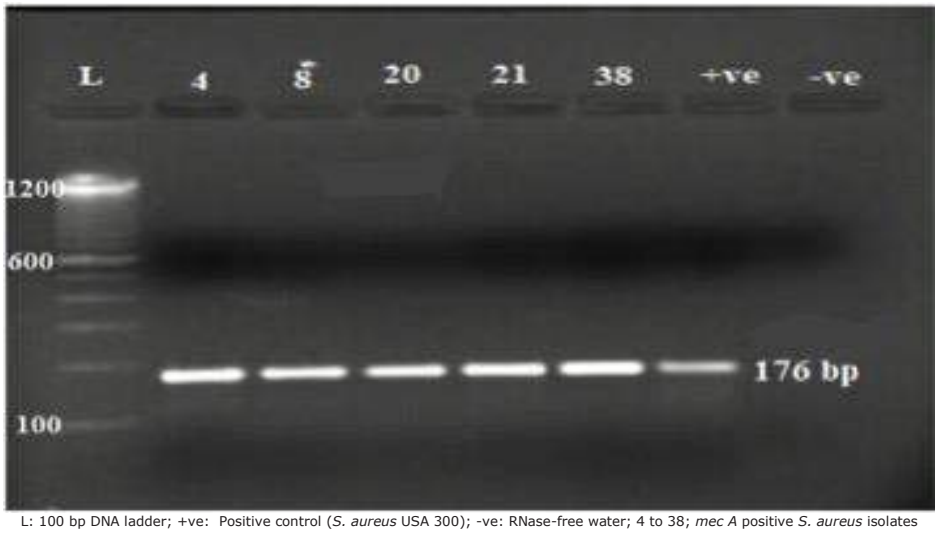
ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; *CHL*-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamycin 10ug; FOX-30: Cefoxitin-30ug; Agogo: Agogo Presbyterian Hospital; KNUST: Kwame Nkrumah University of Science and Technology Hospital; KATH: Komfo Anokye Teaching Hospital.

Fig 2a: Antibiotic susceptibility of methicillin resistant *Staphylococcus aureus* isolates from three healthcare facilities in Ghana



ERY-15: Erythromycin 15 µg; OXA-1: Oxacillin 1 µg; CIP-5: Ciprofloxacin 5 µg; C*HL*-30: Chloramphenicol 30 µg; SXT-25 Trimethoprim-sulphamethoxazole 25ug; TET-30: Tetracycline 30ug; CN-10: Gentamycin 10ug; FOX-30: Cefoxitin-30ug; MRSA: Methicillin resistant *S. aureus*. MSSA: Methicillin sensitive *S. aureus*.

Fig 2b: Comparative antibiotic resistance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from healthcare facilities in Ghana



L: 100 bp DNA ladder; +ve: Positive control (*S. aureus* USA 300); -ve: RNase-free water; 4 to 38; *mec A* positive *S. aureus* isolates

Fig 3: Gel image confirming the 176 bp PCR amplicon of the *mecA* gene in MRSA isolates

Prevalence of resistant genes in multi-drug-resistant *S. aureus* strains:

Of the 68 *S. aureus* isolates, 9 (13.0%) contained *mec A* gene but none possessed *mec* C gene (Fig 3). All the 9 isolates were among the 28 (32.1%) phenotypic MRSA isolates.

Efflux pump activity amongst MDR *S. aureus* isolates:

The MICs of ciprofloxacin and ampicillin for the 25 MDR *S. aureus* tested ranged from 0.8 to 12.8 µg/mL. In the presence of reserpine (an efflux pump inhibitor), a two-fold reduction in the MIC was recorded in 10 of the MDR *S. aureus* isolates and this occurred in 28.6% (n= 8/28) of the MRSA isolates. This reduction in MIC was observed in 10 of 25 (40.0%) isolates tested against ciprofloxacin, 6 of 25 (24.0%) isolates tested against ampicillin, and 6 of 25 (24.0%) isolates tested against both ciprofloxacin and ampicillin, indicating multiple efflux pump activity (Table 4).

Table 4: Effect of efflux pump inhibition (reserpine) on the minimum inhibitory concentration of *Staphylococcus aureus* isolates

MDR *S. aureus* isolate	Minimum inhibitory concentration (µg/mL)					
	Cip alone	Cip+ reserpine	Fold change in MIC	Amp alone	Amp + reserpine	Fold change in MIC
S1	6.4	6.4	1	12.8	12.8	1
S2	6.4	6.4	1	12.8	12.8	1
S3	0.8	0.8	1	12.8	12.8	1
S4	1.6	0.8	2↓	3.2	1.6	2↓
S5	3.2	1.6	2↓	3.2	1.6	2↓
S6	6.4	6.4	1	12.8	12.8	1
S7	3.2	1.6	2↓	6.4	3.2	2↓
S8	1.6	0.8	2↓	6.4	3.2	2↓
S9	0.8	0.8	1	6.4	6.4	1
S10	0.8	0.8	1	12.8	12.8	1
S11	1.6	0.8	2↓	6.4	3.2	2↓
S12	0.8	0.8	1	3.2	3.2	1
S13	0.8	0.8	1	6.4	6.4	1
S14	1.6	0.8	2↓	0.8	0.8	1
S15	6.4	6.4	1	3.2	3.2	1
S16	3.2	3.2	1	3.2	3.2	1
S17	6.4	3.2	2↓	3.2	3.2	1
S18	1.6	1.6	1	6.4	6.4	1
S19	1.6	0.8	2↓	3.2	3.2	1
S20	0.8	0.8	1	6.4	6.4	1
S21	1.6	1.6	1	6.4	6.4	1
S22	3.2	1.6	2↓	12.8	6.4	2↓
S23	0.8	0.8	1	12.8	12.8	1
S24	12.8	12.8	1	12.8	12.8	1
S25	1.6	0.8	2↓	0.8	0.8	1

↓: Reduction in MIC; MIC: minimum inhibitory concentration

Discussion:

Increasing antibiotic resistance among pathogenic bacteria calls for more studies on the prevalence and resistance mechanisms among these bacteria. Continuous monitoring of prevalence, susceptibility patterns and resistance mechanisms of pathogenic bacteria, including MRSA in health facilities is also necessary for effective management and control of bacterial infections (1). Most of the reported surveillance on antimicrobial resistance of pathogens worldwide and in Ghana often involve samples obtained from tertiary or large regional healthcare facilities and teaching hospitals (3,16,17-22) with very few from secondary and primary healthcare facilities. In this study, samples were collected from one regional/teaching hospital and four secondary healthcare facilities including one university hospital and three district hospitals in order to report on antimicrobial resistance at the district levels as well.

In this study, 41.2% of *S. aureus* were identified as MRSA by the cefoxitin disk diffusion test. This value is higher than 28-34.8% previously reported in Ghana (3,16,18). Most studies define MRSA as *S. aureus* isolates resistant to oxacillin, possession of either *mecA* or *mecC* gene or a latex agglutination test for detection of PBP2a (19,20). These could account for the differences seen between these studies. However, in agreement , a review (17) has reported

MRSA prevalence of 23-73% from clinical samples across Europe.

A high number of the MDR-MRSA isolates were identified in the nasal samples (n=10, 35.7%). This relatively high prevalence in nasal samples (nasal carriage of MRSA) could lead to transmission to other individuals visiting the healthcare facilities and within communities (1,22,23). The antibiograms of the *S. aureus* isolates revealed an appreciable level of resistance to the selected antibiotics mostly to oxacillin and cefoxitin (100.0%), tetracycline (82.1%) and chloramphenicol (75.0%). Similarly, high resistance of *S. aureus* to β-lactams (oxacillin) have been reported in Ghana (16,18,20). Half of the isolates tested were however susceptible, to a large extent, to trimethoprim-sulfamethoxazole (50.0%), which is comparable to previously reported studies (21,24).

In this study, *mecA* gene was detected in 9 (32.1%) of the 28 MRSA isolates, representing 13.2% of the total *S. aureus* isolates. A previous study in Ghana reported 28.0% prevalence of *mecA* genes in *S. aureus* isolated from samples from patients at the burns unit of a teaching hospital (22), higher than that observed in our study. The difference could be due to the fact that patients with burns and those on admission in intensive care units tend to be more susceptible to MRSA infections (25). *mecC* genes were not detected in any of the *S. aureus* strains in our study. Globally, the prevalence of *mecC* genes in *S. aureus* is quite low; between 0.4% and 0.7% (24,26,27). *mecC* genes have not yet been reported among clinical *S. aureus* isolates in Ghana.

Aside *mecA* and *mecC* genes, factors such as the presence of multiple efflux pumps have also been identified to contribute to resistance development in *S. aureus* (26,28,29,30), hence the MICs of ciprofloxacin and ampicillin in the presence and absence of an efflux pump inhibitor (reserpine) was used to assess the presence of efflux pump activities among selected isolates not possessing *mecA*. The MICs of ampicillin and ciprofloxacin in the presence of an efflux pump inhibitor (reserpine), reduced by a factor of two in 24.0% and 40.0% of isolates respectively. Similar results of a two-fold reduction in MIC values for norfloxacin in the presence of reserpine has been reported by Dang et al., (27). The reduction in MIC values obtained may signify the presence of multidrug efflux pumps in the MRSA isolates. Efflux pump genes have been found in MRSA isolates from clinical samples in Iran (28,29).

Among the 28 MRSA isolates, 19 (67.9%) had neither *mecA*, *mecC* nor exhibited active efflux pump activity, indicating other mechanisms are responsible for resistance evolution in those MRSA isolates. Studies suggest mutations in the PBP genes and polymorphisms in the *mec* genes could account for such resistance expression. Also, hyper-production/overexpression of β-lactamase antibiotic degrading enzymes could enhance resistance in the MRSA strains (9,27,28). Identification of other possible mechanisms responsible for the multidrug resistance in the *S. aureus* isolates especially in MRSA strains could improve infection management (1).

Conclusion:

The prevalence of *S. aureus* isolates from clinical samples in our study was 10.9%, with 41.2% being phenotypic MRSA isolates. The isolates were least resistant to trimethoprim-sulfamethoxazole among the selected antibiotics. *mecA* gene-mediated-resistance was identified as one of the mechanisms responsible for multidrug resistance in 32.1% of MRSA isolates while *mecC* genes were not detected in the isolates. Active efflux was also identified to be partly responsible for resistance in the isolates.

Contribution of authors:

CNZ, CA, and VEB were in the study conceptualization; CNZ and YDB were involved in data curation; CNZ, HO and VEB were involved in formal analysis: CNZ, YDB and VEB were involved in the study investigation; CNZ, YDB, VEB and CA were involved in the study methodology; CNZ, CA, VEB, and YDB were involved in project administration; CNZ, CA, VEB, and YDB provided resources; CA, YDB and VEB were involved in study supervision; CNZ and HO wrote the original manuscript draft; HO, CNZ, VEB and YDB reviewed and edited the manuscript. All authors reviewed and approved the manuscript submitted for publication.

Source of funding:

No funding was received for the study

Conflict of interest:

Authors declare no conflict of interest

Previous publication:

A previous version of this manuscript was posted as a preprint on Research Square (https://doi.org/10.21203/rs.3.rs-602638/v1) on 22/06/2021

References:

1. Gajdács, M., and Albericio, F. Antibiotic resistance: from the bench to patients. Antibiotics. 2019; 8(3): 129. https://doi.org/10.3390/antibiotics8030129
2. Ippolito, G., and Wenzel, R. P. Methicillin-resistant *Staphylococcus aureus*: the superbug. Int J Infect Dis. 2010;14:7–11. doi: 10.1016/j.ijid.2010.05.003
3. Newman, M. J., Frimpong, E., Donkor, E. S., Opintan, J. A., and Asamoah-Adu, A. Resistance to antimicrobial drugs in Ghana. Infect Drug Resist. 2011; 4: 215-220. doi: 10.2147/IDR.S21769
4. Alkhawam, H., Sogomonian, R., Zaiem, F., et al. Morbidity and mortality of infective endocarditis in a hospital system in New York City serving a diverse urban population. J Investig Med . 2016; 64: 1118–1123. doi: 10.1136/jim-2015-000040.
5. Gajdács, M. The continuing threat of methicillin-resistant *Staphylococcus aureus*. Antibiotics (Basel). 2019;8(2): 52. doi: 10.3390/antibiotics8020052.
6. Becker, K., Ballhausen, B., Köck, R., and Kriegeskorte, A. Methicillin resistance in *Staphylococcus* isolates: The "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol. 2014;304:794–804. doi: 10.1016/j.ijmm.2014.06.007.
7. Pournajaf, A., Ardebili, A., Goudarzi, L., Khodabandeh, M., Narimani, T., and Abbaszadeh, H. PCR-based identification of methicillin–resistant *Staphylococcus aureus* strains and their antibiotic resistance profiles. Asian Pac J Trop Biomed. 2014; 4: S293–S297. doi: 10.12980/APJTB.4.2014C423
8. BA, F. Mec C-harboring methicillin-resistant *Staphylococcus aureus*: hiding in plain sight. J Clin Microbiol. 2018;56:e01549-17. doi: 10.1128/JCM.01549-17
9. Elhassan, M. M., Ozbak, H. A., Hemeg, H. A., Elmekki, M. A., and Ahmed, L. M. Absence of the mecA gene in methicillin resistant *Staphylococcus aureus* isolated from different clinical specimens in Shendi City, Sudan. Biomed Res Int. 2015; 2015: 895860. doi: 10.1155/2015/895860
10. Helsinki WMAD. Ethical Principles for Medical Research Involving Human Subjects. World Med. Assoc. 2018.
11. Karikari, A. B., Frimpong, E., and Owusu-Ofori, A. Methicillin-resistant *Staphylococcus aureus* among patients in a teaching hospital in Ghana. Int J One Heal. 2017; 3: 46–49. doi: 10.14202/ijoh.2017.46-49
12. Dora Bustamante, N. MRSA: A Global Threat. [Texas]: UT Southwestern Medical School; 2011.
13. Odonkor, S. T., Newman, M. J., and Addo, K. K. Prevalence and antibiotic susceptibility profile of methicillin resistant *Staphylococcus aureus* in Accra, Ghana. Microbiol Res (Pavia). 2012; 3: 20.
14. Odonkor, S. T., and Addo, K. K. Evaluation of Three Methods For Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). BioMed Sci Direct. 2011; 2: 1031–1034.
15. Pu, W., Su, Y., Li, J., et al. High Incidence of Oxacillin-Susceptible mecA-Positive *Staphylococcus aureus* (OS-MRSA) Associated with Bovine Mastitis in China. Zhang Q, editor. PLoS One. 2014;9:e88134. doi: 10.1371/journal.pone.0088134.
16. Huse, H. K., Miller, S. A., Chandrasekaran, S., et al. Evaluation of Oxacillin and Cefoxitin Disk Diffusion and MIC Breakpoints Established by the Clinical and Laboratory Standards Institute for Detection of mecA-Mediated Oxacillin Resistance in *Staphylococcus schleiferi*. J Clin Microbiol. 2017; 56: e01653-17. doi: 10.1128/JCM.01653-17
17. Wu, M. T., Burnham, C-AD., Westblade, L. F., et al. Evaluation of Oxacillin and Cefoxitin Disk and MIC Breakpoints for Prediction of Methicillin Resistance in Human and Veterinary Isolates of *Staphylococcus* intermedius Group. J Clin Microbiol. 2016; 54: 535–542. doi: 10.1128/JCM.02864-15
18. Bard, J. D., Hindler, J. A., Gold, H. S., and Limbago, B. Rationale for eliminating *staphylococcus* breakpoints for β-lactam agents other than penicillin, oxacillin or cefoxitin, and ceftaroline. Clin Infect Dis. 2014; 58: 1287–1296. doi: 10.1093/cid/ciu043.
19. Amissah, N. A., Buultjens, A. H., Ablordey, A., et al. Methicillin resistant *Staphylococcus aureus* transmission in a Ghanaian burn unit: The importance of active surveillance in resource-limited settings. Front Microbiol. 2017; 8: 1906. doi: 10.3389/fmicb.2017.01906
20. Egyir, B., Guardabassi, L., Sørum M., et al. Molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. PLoS One. 2014;9:e89716. https://doi.org/10.1371/journal.pone.0089716
21. Egyir, B., Guardabassi, L., Monecke, S., Addo, K. K., Newman, M. J., and Larsen, A. R. Methicillin-resistant *Staphylococcus aureus* strains from Ghana include USA300. J Glob Antimicrob Resist. 2015;3:26–30. doi: 10.1016/j.jgar.2014.11.006
22. Amissah, N. A., Dam, L. V., Ablordey, A., et al. Epidemiology of *Staphylococcus aureus* in a burn unit of a tertiary care center in Ghana. PLoS One. 2017;12:e0181072. https://doi.org/10.1371/journal.pone.0181072
23. Reygaert, W. C. Antimicrobial resistance mechanisms of *Staphylococcus aureus*. Microb Pathog Strateg Combat them Sci Technol Educ. 2013: 297–305.
24. Kerschner, H., Harrison, E. M., Hartl, R., Holmes, M. A., and Apfalter, P. First report of mecC MRSA in human samples from Austria: molecular characteristics and clinical data. New Microbes New Infect. 2015; 3: 4–9. https://doi.org/10.1016/j.nmni.2014.11.001
25. Paterson, G. K., Harrison, E. M., and Holmes, M. A. The emergence of mecC methicillin-resistant *Staphylococcus aureus.* Trends Microbiol. 2014;22: 42–47. doi: 10.1016/j.tim.2013.11.003
26. Jang, S. Multidrug efflux pumps in *Staphylococcus aureus* and their clinical implications. J Microbiol. 2016;54:1–8. doi: 10.2174/1874285801307010059
27. Dang, T. N. D., Srinivasan, U., Britt, Z., et al. Efflux-mediated Resistance Identified Among Norfloxacin Resistant Clinical Strains of Group B *Streptococcus* From South Korea. Epidemiol Health. 2014; 36: e2014022. doi: 10.4178/epih/e2014022
28. Dashtbani-Roozbehani, A., and Brown, M. H. Efflux pump mediated antimicrobial resistance by *staphylococci* in health-related environments: Challenges and the quest for inhibition. Antibiotics. 2021; 10 (12): 1502. doi: 10.3390/antibiotics10121502
29. Bayat, B., Zade, M. H., Mansouri, S., et al. High frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) with SCCmec type III and spa type t030 in Karaj's teaching hospitals, Iran. Acta Microbiol Immunol Hung. 2017;64 (3): 331-341. doi: 10.1556/030.64.2017.020

Ukpai & Onah. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 297 - 308 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2516. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Prevalence and antibiotic susceptibility profiles of methicillin-resistant *Staphylococcus aureus* isolates from apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria

*[1]Ukpai, E. G., and [2]Onah, E. S.

[1]Department of Microbiology and Parasitology, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
[1]International Institute of Infectious Diseases, Biosafety and Biosecurity Research, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
[2]Department of Ophthalmology, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
[2]Institute for Eye Health and Visual Science Research (IEHVSR), David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
*Correspondence to: ukpaieg@dufuhs.edu.ng

Abstract:

Background: Infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) remains a big health challenge around the world because it is associated with increase morbidity, mortality and healthcare costs. This study aimed to determine the prevalence and antibiotic susceptibility profiles of MRSA isolated from apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria.
Methodology: This was a descriptive cross-sectional study of apparently healthy pupils and students from the three Senatorial districts in each of Enugu and Ebonyi States, recruited by multi-stage random sampling technique. Urine and swab samples of nose, armpit and groin were collected from the participants and cultured for *Staphylococcus aureus* using standard microbiological methods. MRSA isolates were detected by the Kirby-Bauer disc diffusion method using cefoxitin 30µg disc. Statistical analysis of data was done using Chi-square and Fisher's exact tests, and binary logistic regression analysis to compare the prevalence of *S. aureus* and MRSA with respect to gender, schools, senatorial districts, and States. P value less than 0.5 was considered statistical significance.
Results: A total of 441 participants were recruited with 234 (53.1%) males and 207 (46.9%) females. Samples of 206 (46.7%) participants yielded *S. aureus,* and 140 (31.7%) were confirmed as MRSA. Gender distribution showed comparable *S. aureus* colonization rate between males (47.0%, n=110) and females (46.4%, n=96), while the prevalence of MRSA colonization was higher in females (35.3%, n=73) than males (28.6%, n=67). State-specific prevalence of MRSA colonization was 30.7% (n=71/231) in Enugu and 32.9% (n=69/210) in Ebonyi. There was no statistically significant association between MRSA colonization prevalence and sex (χ^2=2.89, p=0.089) or State (χ^2=0.46, p=0.50). However, prevalence of MRSA colonization was higher among secondary school students compared to primary school pupils, with borderline statistical significance (χ^2=3.84, p=0.050). The overall antibiotic resistance profile of MRSA isolates revealed resistance rates ranging from 15.1% for gentamicin to 97.9% for penicillin.
Conclusion: This study reported high prevalence of MRSA colonization among apparently healthy pupils and students in both Enugu and Ebonyi States, southeast Nigeria. Government should strengthen infection prevention efforts in schools and enforce surveillance policies for antimicrobial resistance in the States. Antibiotics such as ciprofloxacin and gentamicin may still be effective for therapy of MRSA infections in the region.

Keywords: Prevalence; Antibiotic susceptibility; MRSA; Colonization; Pupils; Students

Received Apr 21, 2026; Revised (expedited) May 5, 2026; Accepted May 6, 2026

Prévalence et profils de sensibilité aux antibiotiques des isolats de *Staphylococcus aureus* résistant à la méthicilline chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au Nigéria

*[1]Ukpai, E. G., et [2]Onah, E. S.

[1]Département de Microbiologie et de Parasitologie, Université Fédérale des Sciences de la Santé David Umahi, Uburu, État d'Ebonyi, Nigéria
[1]Institut International de Recherche sur les Maladies Infectieuses, la Biosécurité et la Biosûreté, Université Fédérale des Sciences de la Santé David Umahi, Uburu, État d'Ebonyi, Nigéria
[2]Département d'Ophtalmologie, Université Fédérale des Sciences de la Santé David Umahi, Uburu, État d'Ebonyi, Nigéria
[2]Institut de Recherche sur la Santé Oculaire et les Sciences de la Vision (IEHVSR), Université Fédérale des Sciences de la santé David Umahi, Uburu, État d'Ebonyi, Nigéria
*Correspondance à: ukpaieg@dufuhs.edu.ng

Résumé:

Contexte: L'infection à *Staphylococcus aureus* résistant à la méthicilline (SARM) demeure un problème de santé publique majeur à l'échelle mondiale, car elle est associée à une augmentation de la morbidité, de la mortalité et des coûts des soins de santé. Cette étude visait à déterminer la prévalence et les profils de sensibilité aux antibiotiques du SARM isolé chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au Nigéria.
Méthodologie: Il s'agit d'une étude descriptive transversale menée auprès d'élèves et étudiants apparemment en bonne santé issus des trois districts sénatoriaux de chacun des États d'Enugu et d'Ebonyi, recrutés par échantillonnage aléatoire à plusieurs degrés. Des échantillons d'urine et des prélèvements nasaux, axillaires et inguinaux ont été collectés chez les participants et mis en culture pour la recherche de Staphylococcus aureus selon les méthodes microbiologiques standard. Les isolats de SARM ont été détectés par la méthode de diffusion sur disque de Kirby-Bauer, en utilisant un disque de céfoxitine 30µg. L'analyse statistique des données a été réalisée à l'aide des tests du χ^2 et de Fisher, ainsi que d'une régression logistique binaire, afin de comparer la prévalence de *S. aureus* et du SARM en fonction du sexe, de l'établissement scolaire, de la circonscription sénatoriale et de l'État. Un seuil de signification statistique de $p<0,05$ a été retenu.
Résultats: Au total, 441 participants ont été recrutés, dont 234 hommes (53,1%) et 207 femmes (46,9%). Des prélèvements ont révélé la présence de *S. aureus* chez 206 participants (46,7%), et 140 (31,7%) ont été confirmés comme porteurs du SARM. La répartition par sexe a montré un taux de colonisation par *S. aureus* comparable entre les hommes (47,0%, n=110) et les femmes (46,4%, n=96), tandis que la prévalence de la colonisation par le SARM était plus élevée chez les femmes (35,3%, n=73) que chez les hommes (28,6%, n=67). La prévalence de la colonisation par le SARM était de 30,7% (n=71/231) à Enugu et de 32,9% (n=69/210) à Ebonyi. Aucune association statistiquement significative n'a été observée entre la prévalence de la colonisation par le SARM et le sexe ($\chi^2=2,89$, $p=0,089$) ou l'État ($\chi^2=0,46$, $p=0,50$). Cependant, la prévalence de la colonisation par le SARM était plus élevée chez les élèves du secondaire que chez ceux du primaire, avec une signification statistique limite ($\chi^2=3,84$, $p=0,050$). Le profil global de résistance aux antibiotiques des isolats de SARM a révélé des taux de résistance allant de 15,1% pour la gentamicine à 97,9% pour la pénicilline.
Conclusion: Cette étude a mis en évidence une forte prévalence de la colonisation par le SARM chez des élèves et étudiants apparemment en bonne santé dans les États d'Enugu et d'Ebonyi, au sud-est du Nigéria. Les autorités devraient renforcer les efforts de prévention des infections dans les écoles et appliquer des politiques de surveillance de la résistance aux antimicrobiens dans ces États. Des antibiotiques comme la ciprofloxacine et la gentamicine peuvent encore être efficaces pour le traitement des infections à SARM dans la région.

Mots-clés: Prévalence; Sensibilité aux antibiotiques; SARM; Colonisation; Élèves; Étudiants

Introduction:

Staphylococcus aureus remains one of the most clinically important bacterial pathogens worldwide due to its remarkable capacity to colonize humans, cause a wide range of infections, and rapidly acquire antimicrobial resistance (1,2). The emergence and global spread of methicillin-resistant *S. aureus* (MRSA) have significantly altered the epidemiology and clinical management of *S. aureus* infections over the last five decades. Initially confined to hospital settings in the 1960s and 1970s, MRSA evolved into a major cause of healthcare-associated infections (HA-MRSA), particularly among hospitalized and immunocompromised individuals (3,4). However, a major epidemiological shift occurred in the mid-1990s when MRSA strains began to emerge in individuals with no prior healthcare exposure, giving rise to MRSA in settings outside healthcare (5-8).

Staphylococcus aureus commonly exists as a normal flora on the skin and in the nasal passages of healthy individuals, yet it possesses the capacity to cause infections ranging from minor skin conditions to serious systemic diseases (6-8). Its silent colonization makes it a major source of transmission within communities. This is especially heightened among children and adolescents, who often interact closely in crowded settings like schools, promoting its spread (9-12). Although it is not a typical cause of urinary tract infections, its presence in urine especially among apparently healthy individuals may indicate colonization or transient carriage with potential clinical and epidemiolo-

gical significance (13).

In Africa, MRSA epidemiology is complex and underreported, with significant regional variations. Studies across West, East, and Southern Africa consistently reveal increasing prevalence of MRSA in both hospitals and community settings, fueled by widespread antibiotic misuse, poor infection control practices, overcrowding, and limited surveillance systems (14, 15). Nigeria, in particular, bears a substantial burden of *S. aureus* infections and has reported high and rising MRSA prevalence across various States and population groups. Previous research from southeastern Nigeria shows that MRSA colonization is widespread among school children, adults, hospital outpatients, livestock handlers, and apparently healthy community members (16-17). This highlights the expanding reservoir of MRSA and the increasing risk of transmission within public institutions, including schools.

School environments represent one of the most important settings for MRSA transmission due to overcrowding, frequent skin-to-skin contact, limited access to hygiene facilities, and behavioral factors common among children and adolescents such as sharing of personal belongings (11,18-20). Several studies indicate that primary school pupils may be more vulnerable to MRSA colonization because of poor hygiene practices, increased physical interaction, and susceptibility to minor skin injuries that facilitate bacterial colonization (6). Gender-related differences also exist, with some research reporting higher MRSA colonization among females, possibly due to grooming habits, sharing of personal items, or cultural practices that enhance bacterial dissemination (21, 22). The occurrence of *S. aureus* in urine samples of asymptomatic individuals is increasingly recognized as an important public health concern (18,23). Such carriage may serve as a silent reservoir for transmission and may predispose individuals to future infections under favorable conditions (24,25). Hence, surveillance in this population is essential for timely detection and outbreak prevention.

The detection of MRSA in urine, groin, armpit anterior nares and wound samples from apparently healthy school pupils and students raises concerns about the hidden burden of antimicrobial resistance within the community. The southeastern region of Nigeria, particularly Enugu and Ebonyi States, has experienced rising incidences of antibiotic-resistant infections over the last decade. Despite this, data specifically focusing on urinary carriage among asymptomatic school populations, particularly in Enugu and Ebonyi States, remain limited. Generating such data is crucial for understanding local epidemiology and informing control strategies.

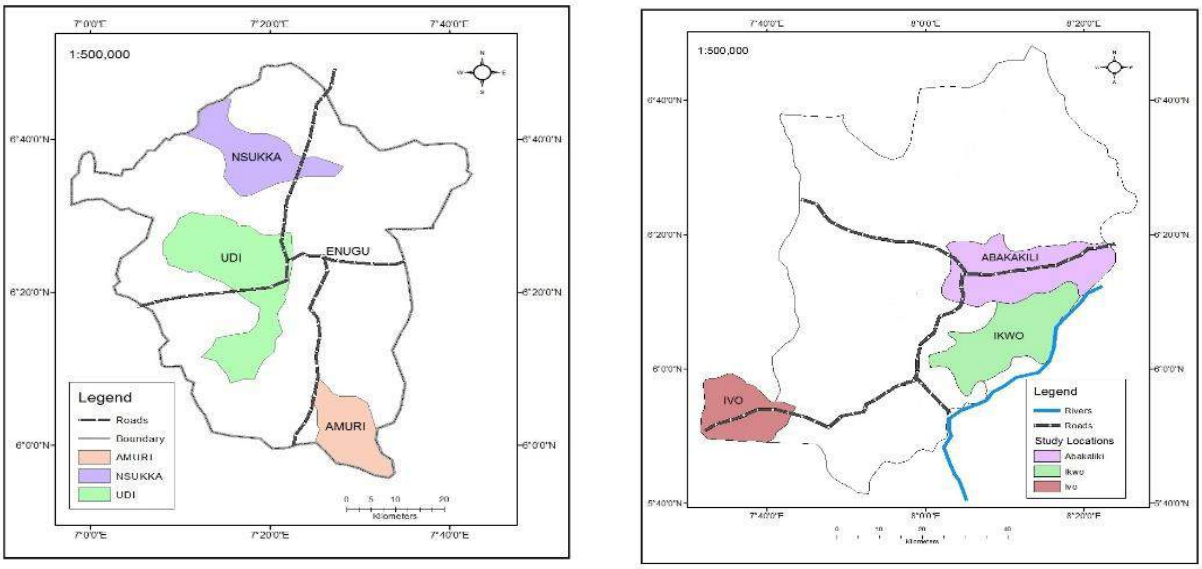
This study therefore aimed to determine the prevalence and antibiotic susceptibility patterns of methicillin-resistant *S. aureus* isolated from samples of apparently healthy school pupils and students in Enugu and Ebonyi States, Nigeria. The findings are expected to contribute to existing knowledge and support the development of effective infection prevention measures and rational antibiotic use policies.

Materials and method:

Study design and setting:

This was a descriptive cross-sectional study of apparently healthy selected pupils and students carried out in Enugu and Ebonyi States, located in the southeastern region of Nigeria (Fig 1a and b). Enugu State lies between 5°56′N–7°06′N and 6°53′E–7°55′E. It has a tropical climate with wet and dry seasons that support microbial persistence. The State is largely urban, especially in Enugu, with high population density and frequent interpersonal contact, facilitating *S. aureus* and MRSA transmission. Numerous schools and healthcare facilites, including the University of Nigeria Teaching Hospital, provide a suitable setting for studying bacterial colonization and antimicrobial resistance patterns (27).

Ebonyi State lies between 5°40′N–6°45′N and 7°30′E–8°30′E. It has a tropical climate with significant rainfall supporting agriculture. The State is predominantly rural, with Abakaliki as the main urban center. Farming and livestock rearing increase human–animal contact, influencing *S. aureus* transmission. Limited healthcare access and variable sanitation may affect resistance patterns. Facilities such as the Alex Ekwueme Federal University Teaching Hospital support healthcare delivery and research (28).



(a) (b)

Fig 1: Geographical Map of Enugu State (1a) and Ebonyi State (1b) showing the study locations in the three different senatorial study location zones (26)

Study participants and inclusion criteria:

For this study, participants were apparently healthy pupils and students aged 8-12 years and 12-18 years, respectively. Apparently healthy individuals were defined as participants who, upon brief clinical assessment, exhibited no signs of active infection (e. g. no fever ≥38°C, no pain or disability, abscesses, or nasal discharge), had no recent (≤3 months) hospitalization, surgery, or invasive medical procedures, no antibiotic use within the preceding 2–4 weeks, and no known immunocompromising conditions.

Eligibility was further supported by participant self-report and, where applicable, review of medical history. Participants were selected from representative schools (primary and secondary) from each of the three senatorial districts in both States i. e. three primary and three secondary schools from each State.

Ethical consideration:

Informed consent was obtained from the parents or legal guardians of all participants below 18 years of age after providing detailed information about the study objectives, procedures, potential risks, and benefits. In addition to parental/guardian consent, assent was obtained from adolescents aged 12–17 years after the study was explained in age-appropriate language. These participants were informed that their participation was voluntary and that they could decline participation or withdraw at any stage without penalty.

For younger children below 12 years of age, the study procedures were explained in simple and understandable terms, and verbal assent was obtained where appropriate before sample collection. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material or data (29).

Determination of sample size:

The sample size for the study was calculated using the single population proportion formula, $n = Z^2p(1-p)/d^2$ where, n = required sample size, Z=95% confidence level (=1.96), p = estimated prevalence of 45.6% (=0.456) from a previous study (8), and d=margin of error of 5% (0.05). Thus, applying these parameters, the minimum sample size required for this study was calculated as 381. However, a sample size of 441 was considered adequate to compensate for non-response or sample loss and to improve statistical power.

Sampling technique:

The study area covers two States (Enugu and Ebonyi) hence, a multistage sampling method was applied to systematically select participants in stages. Stage 1 involved selection of senatorial districts or local government areas (LGAs) within each State; stage 2 involved selection of schools (primary and secondary) within each selected LGA; and stage 3 involved selection of pupils and students within the selected schools. This approach was to ensure a good geographical coverage and to

make the study more practical and cost-effective.

Secondly, within the selected schools, stratified random sampling was used to stratify participants based on factors including age group (8–12 years and 12–18 years), educational level (primary vs secondary school) and gender. A simple random sampling was applied within each stratified group to select participants, which ensured that all relevant subgroups were adequately represented in the study.

Sample and data collection:

Clinical samples were randomly collected from a total of 441 pupils and students (234 males, and 207 females), with urine (n= 398), wound swab (n=15), armpit swab (n= 11), nasal swab (n=11), and groin swab (n= 6). The demographic data (age, sex) of the participants were collected using a designed proforma.

Clean-catch midstream freshly voided urine specimens were collected into sterile plastic specimen bottles. The samples were transported to the Department of Applied Microbiology Laboratory unit, Faculty of Science, Ebonyi State University, Abakaliki within two hours of collection for bacteriological analysis.

Isolation and identification of *Staphylococcus aureus* isolates:

Samples were aseptically inoculated on Mannitol Salt Agar (Oxoid, United Kingdom) and incubated aerobically at 37°C for 18–24 hours. Discrete colonies were sub-cultured on fresh MSA plates to obtain pure isolates. Presumptive *S. aureus* isolates were identified based on the appearance of yellow or golden colonies with mannitol fermentation on MSA. Further confirmation was carried out using standard microbiological procedures, including Gram staining, catalase test, coagulase test, haemolysis on blood agar, and assessment of colony morphology (30,31).

Isolation of *S. aureus* and MRSA from urine samples was considered clinically significant only when recovered as a pure culture at $\geq 10^5$ CFU/ml, or at $\geq 10^4$ CFU/mL with supportive evidence such as pyuria or repeat isolation, to minimize the inclusion of contaminants from skin flora.

Antibiotic susceptibility test of *Staphylococcus aureus* isolates:

The antibiotic susceptibility test of the identified isolates was performed by the disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. Inoculum of the test isolate was prepared from pure colonies on the sub-culture plate and standardized to 0.5 McFarland standard equivalent, which was then inoculated on the surface of Mueller Hinton agar (Oxoid, UK) plates using sterile swab stick.

Antibiotic discs (Oxoid UK); ceftazidime (CAZ, 30µg), ciprofloxacin (CIP, 5µg), clindamycin (DA, 2µg), penicillin (P, 10µg), erythromycin (E, 15µg), nitrofurantoin (F_{200}, 300µg), gentamicin (CN, 5µg), sulphamethoxazole (RL, 25µg), and tetracycline (TE, 30µg) were placed on the surface of the inoculated agar plates 30mm apart using a sterile forcep. The antibiotics were allowed to diffuse for about 10 minutes and the plates incubated at 37°C for 18-24 hours. The diameters of inhibition zones were measured in millimeter with a meter rule, recorded and interpreted as sensitive or resistant according to the Clinical Laboratory Standard Institute (CLSI) guidelines (32).

Detection of methicillin resistant *S. aureus:*

This was done using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Pure colonies of the isolated bacteria were inoculated into 5 ml nutrient broth. The turbidity of the broth was adjusted to 0.5 McFarland standards and inoculated on Mueller-Hinton agar plate with a sterile swab. Cefoxitin (FOX, 30µg) disc was placed on the inoculated agar plates to detect MRSA. The plates were incubated at 37°C for full 24 hours. Diameter of zone of inhibition was measured, recorded and interpreted as resistant if the inhibition zone diameter was ≤ 21 mm, according to the CLSI guidelines (32).

Determination of multiple antibiotic resistance index (MARI) of MRSA isolates:

Multiple antibiotic resistance index (MA RI) of each *S. aureus* isolate was calculated as the number of antibiotics to which the isolate was resistant to, divided by the total number of antibiotics that was tested on the isolate, as previously described by Ukpai et al., (31).

Statistical analysis of data:

The prevalence of *S. aureus* and MRSA colonization across the States, schools, and gender was compared using the Chi-square test of independence. Fisher's exact test was applied where expected cell counts were less than five. A p-value<0.05 was considered statistically significant. Additionally, binary logistic regression analysis was performed to identify factors associated with MRSA colonization.

Results

Prevalence of *Staphylococcus aureus* and MRSA colonization among the study participants:

Table 1 shows the distribution of *S. aureus* and MRSA isolates among the participants across the senatorial districts in both Enugu and Ebonyi States. The overall prevalence of *S. aureus* colonization is 46.7%, while the overall prevalence for MRSA is 31.7%. Ebonyi State showed a higher prevalence of both *S. aureus* (49.5%) and MRSA (32.9%) compared to Enugu, with 44.2% and 30.7%, respectively, indicating a marginally higher but insignificant colonization rate of pupils and students in Ebonyi State by *S. aureus* and MRSA (p=0.3017 and p=0.7073).

Although *S. aureus* colonization rate was similar between males and females in both States (p=0.9916 for Ebonyi, p=0.9849 for Enugu, and p=0.9704 for both States), MRSA prevalence was consistently but insignificantly higher among females in both States (p= 0.2849 for Ebonyi, p=0.4424 for Enugu, and p=0.1642 for both States), suggesting possible gender-related exposure and behavioral factors influencing transmission.

Comparative prevalence of *S. aureus* and MRSA colonization among the study participants:

The prevalence distribution of *S. aureus* and MRSA colonization among the participants across school types is shown in Table 2. In Enugu, secondary school students had significantly higher prevalence of *S. aureus* (57.8%) and MRSA (42.0%) colonization than primary school pupils (34.8% and 18.8%) (p=0.0083 and p=0.0002, respectively), whereas in Ebonyi, primary school pupils had higher but insignificant *S. aureus* (53.3%) and MRSA (38.1%) colonization compared to secondary school students (45.7% and 27.6%) (p=0.3340 and p= 0.1418, respectively).

Overall, secondary school students had higher but insignificant MRSA colonization rate (35.3%) than primary schools (28.1%) (p= 0.1305). These differences suggest that environmental factors, hygiene practices, or age-related behaviors may influence MRSA transmission differently in each State.

Table 1: Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* colonization among apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

States	**Total no examined**		**No +ve for *S. aureus* (%)**		**No +ve for MRSA (%)**	
	M	**F**	**M**	**F**	**M**	**F**
Enugu State						
SENE	26	14	9 (34.6)	6 (42.8)	2 (7.6)	4 (28.6)
PENE	18	24	8 (44.4)	2 (8.3)	4 (22.2)	1 (4.2)
SENN	28	12	11 (39.3)	8 (66.7)	11 (39.3)	8 (66.7)
PENN	15	12	5 (33.3)	1 (8.3)	5 (33.3)	1 (8.3)
SENS	13	26	11 (84.6)	18 (69.2)	8 (61.5)	17 (65.3)
PENS	21	22	10 (47.6)	13 (59.1)	4 (19.0)	6 (27.3)
Sub-Total	121	110	54 (44.6)	48 (43.6)	34 (28.1)	37 (33.6)
	231		102 (44.2)		71 (30.7)	
Ebonyi State						
SEBN	14	10	4 (28.6)	2 (20.0)	0	1 (10.0)
PEBN	22	18	7 (31.8)	6 (33.3)	5 (22.7)	5 (35.7)
SEBC	33	19	21 (63.6)	11 (57.9)	12 (36.4)	9 (47.4)
PEBC	17	13	10 (58.8)	7 (53.9)	5 (29.4)	3 (23.1)
SEBS	9	20	2 (22.2)	8 (40.0)	1 (11.1)	6 (30.0)
PEBS	18	17	12 (66.7)	14 (82.4)	10 (55.6)	12 (70.6)
Sub-Total	113	97	56 (49.6)	48 (49.5)	33 (29.2)	36 (37.1)
	210		104 (49.5)		69 (32.9)	
Total	**234**	**207**	**110 (47.0)**	**96 (46.4)**	**67 (28.6)**	**73 (35.3)**
	441		**206 (46.7)**		**140 (31.7)**	

SENE- Secondary school Enugu East Senatorial Zone PENE- Primary school Enugu East Senatorial Zone
SENN- Primary school Enugu North Senatorial Zone. PENN- Primary school Enugu North Senatorial Zone
SENS- Secondary school Enugu South Senatorial Zone. PENS-Primary school Enugu South Senatorial Zone
SEBN- Secondary school Ebonyi North Senatorial Zone. PEBN- Primary school Ebonyi North Senatorial Zone
SEBC- Secondary school Ebonyi North Senatorial Zone. PEBC- Primary school Ebonyi North Senatorial Zone
SEBS -Secondary school Ebonyi North Senatorial Zone. PEBS- Primary school Ebonyi North Senatorial Zone

Table 2: Comparative prevalence distribution of *Staphylococcus aureus* and MRSA colonization among apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

Location	Total no examined		No positive for *S. aureus* (%)		No positive for MRSA (%)	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
Enugu	112	119	39 (34.8)	63 (57.8)	21 (18.8)	50 (42.0)
Ebonyi	105	105	56 (53.3)	48 (45.7)	40 (38.1)	29 (27.6)
Total	**217**	**224**	**95 (43.8)**	**111 (49.6)**	**61 (28.1)**	**79 (35.3)**

Antibiotic resistance profile of MRSA isolates:

The antibiotic resistance profile (Table 3, Fig 2 and Fig 3), supported by the MARI distribution (Table 4), indicates widespread multi-drug resistance among the MRSA isolates. Very high resistance rates were observed for penicillin (97.9%), ceftazidime (93.0%), and sulph amethoxazole (90.7%), while moderate resistance was seen with erythromycin (76.8%), tetracycline (75.7%), and clindamycin (68.7%). In contrast, lower resistance rates were recorded for gentamicin (15.1%) and ciprofloxacin (23.3%).

The predominance of high MARI values (≥0.6) in both States, especially in Ebonyi indicates high antimicrobial consumption in the community.

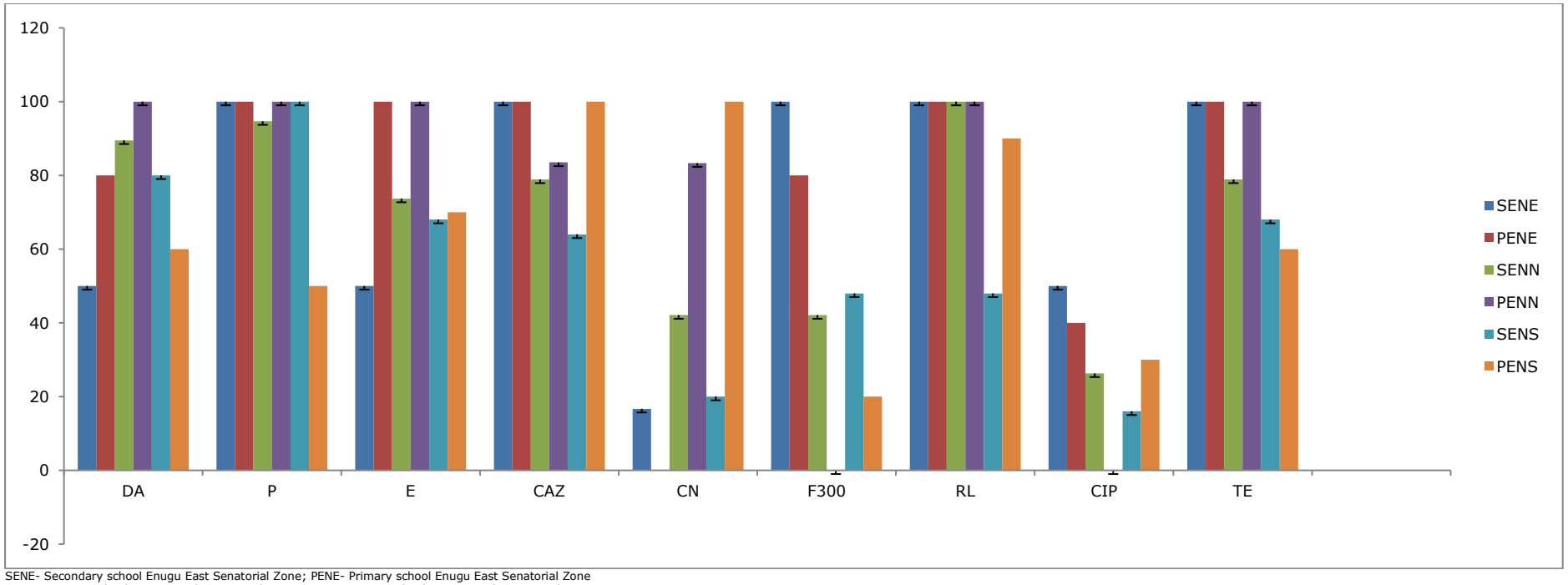
Table 4: Multiple Antibiotic Resistance Index (MARI) of MRSA isolates from apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

MARI value	Enugu	Ebonyi
	No of MRSA (%)	No of MRSA (%)
0.1	3 (4.2)	2 (2.9)
0.2	2 (2.8)	2 (2.9)
0.3	3 (4.2)	6 (8.5)
0.4	12 (16.9)	5 (7.3)
0.5	5 (7.0)	6 (8.5)
0.6	11 (15.5)	10 (14.5)
0.7	11 (15.5)	16 (23.2)
0.8	12 (16.9)	17 (24.6)
0.9	12 (16.9)	5 (7.3)
1.0	0	0
Total	**71 (100)**	**69 (100)**

No (%) = Number and percentage of MRSA with specified MARI value

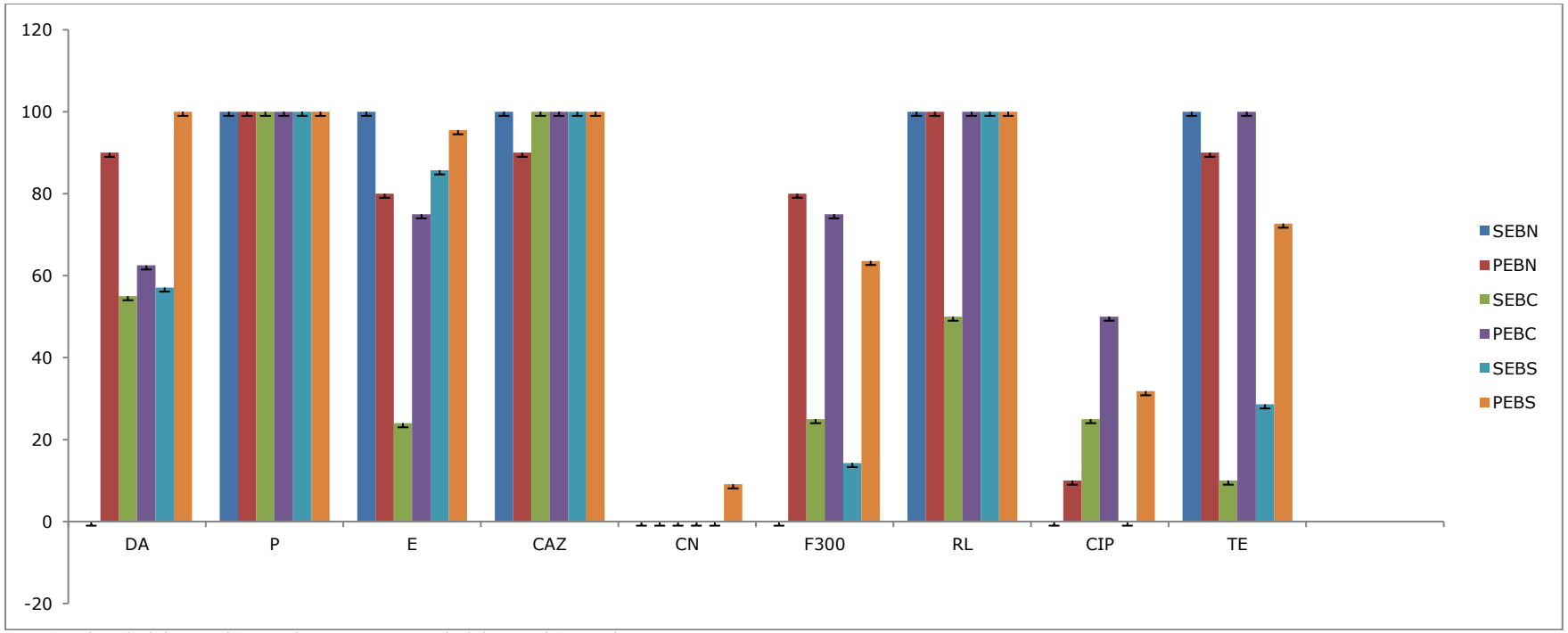
Table 3: Antibiotic resistance (%) of MRSA isolates from apparently healthy pupils and students in Enugu and Ebonyi States, Nigeria

Antibiotics	MRSA isolates		
	Enugu (%)	Ebonyi (%)	Average of both States (%)
Penicillin	95.6	100.0	97.9
Ceftazidime	87.7	98.3	93.0
Sulphamethoxazole	89.7	91.7	90.7
Erythromycin	76.8	76.7	76.8
Clindamycin	76.6	60.8	68.7
Nitrofurantoin	48.4	43.0	45.7
Ciprofloxacin	27.1	19.5	23.3
Gentamicin	28.7	1.5	15.1
Tetracycline	84.5	66.9	75.7



SENE- Secondary school Enugu East Senatorial Zone; PENE- Primary school Enugu East Senatorial Zone
SENN- Primary school Enugu North Senatorial Zone; PENN- Primary school Enugu North Senatorial Zone
SENS- Secondary school Enugu South Senatorial Zone; PENS-Primary school Enugu South Senatorial Zone
DA=Clindamycin; P=Penicillin; E= Erythromycin; CAZ= Ceftazidime; CN= Gentamicin; F_{200} = Nitrofurantoin;
RL=Sulphamethoxazole; CIP= Ciprofloxacin; TE= Tetracyline

Fig 2: Percentage antibiotic resistance pattern of MRSA isolated from schools (secondary and primary) in Enugu State, Nigeria



SEBN- Secondary school Ebonyi North Senatorial Zone. PEBN- Primary school Ebonyi North Senatorial Zone
SEBC- Secondary school Ebonyi North Senatorial Zone. PEBC- Primary school Ebonyi North Senatorial Zone
SEBS -Secondary school Ebonyi North Senatorial Zone. PEBS- Primary school Ebonyi North Senatorial Zone
DA=Clindamycin; P=Penicillin; E= Erythromycin; CAZ= Ceftazidime; CN= Gentamicin; F_{200} = Nitrofurantoin;
RL=Sulphamethoxazole; CIP= Ciprofloxacin; TE= Tetracyline

Fig 3: Percentage antibiotic resistance pattern of MRSA isolated from schools (secondary and primary) in Ebonyi State, Nigeria

Discussion:

The burden of MRSA has been on the increase over the years despite several efforts. Although the overall prevalence of MRSA colonization is 31.7% in this study, the distribution varied significantly across senatorial zones in both Enugu and Ebonyi States. In Enugu State, MRSA prevalence was lowest in Enugu East (13.4%) and markedly higher in Enugu North (37.3%) and Enugu South (42.7%), and this difference was statistically significant (p<0.001). Similarly, in Ebonyi State, MRSA prevalence differed significantly across senatorial zones, with the lowest prevalence in Ebonyi North (17.2%), compared to Ebonyi Central (35.4%) and Ebonyi South (45.3%) (p=0.002). Overall, these findings indicate a significant geographical variation in MRSA colonization across senatorial zones, with consistently higher prevalence observed in southern zones of both States.

The results of this study demonstrate a high overall comparative prevalence of MRSA in both Enugu and Ebonyi States. However, the findings consistently reveal a greater burden in Ebonyi State compared to Enugu. Specifically, Ebonyi State recorded a prevalence of 32.9%, which exceeds the 30.7% reported in Enugu State. Ebonyi also had a higher *S. aureus* carriage rate (49.5%) compared with Enugu (44.2%), which expands the reservoir from which MRSA emerges. Similar *S. aureus* carriage rates have been reported by Abdullahi et al., (33) (31.5%) Rosal et al., (34) (33.0%); Ogefere et al., (35) (42.3%) and Akerele et al., (36) (49.5%).

The prevalence of MRSA reported in some previous studies by Okwu et al., (37) 6.0%; Rosal et al., (34) 4.4%; and Akerele et al., (36) 22.2% are lower than the rate reported in this study. In contrast, higher prevalence rates have been reported by Morufat et al., (18) 45.6%; Azzam et al., (38) 42.2%; Abdullahi et al., (33) 23.5% and Ogefere et al., (35) 37.2%. Furthermore, this pattern aligns with research suggesting that rural and peri-urban populations with limited hygiene infrastructure and reduced access to regulated healthcare practices often experience higher community MRSA transmission (39,40). Thus, the data indicate that Ebonyi State bears a heavier overall burden.

Gender-based analysis revealed notable differences in MRSA colonization, with females exhibiting higher carriage rates than males in both States. In Enugu State, MRSA prevalence was 33.6% among females compared to 28.1% among males, while in Ebonyi State, females had a prevalence of 37.1% versus 29.2% in males. These findings are consistent with previous reports indicating higher MRSA colonization among females, which have been attributed to behavioral factors such as sharing of personal items, close interpersonal contact and frequent grooming-related practices that enhance transmission (21,22,35,41).

However, some studies have reported male predominance in MRSA colonization in other settings, including those by Kodde et al., (42) in Iran, Rosal et al., (34) in Spain, and Akerele et al., (36) in Edo State, Nigeria. In this study, there was a statistically significant association of the prevalence of *S. aureus* and the female gender of the participants (p=0.02). However, there was no statistically significant association in the prevalence of MRSA between males and females in Enugu and Ebonyi States (p=0.051). The observed pattern in southeastern Nigeria may reflect the prevailing social and behavioral interactions among female pupils and adolescents within school environments.

Educational level comparisons further reveal important distinctions in MRSA colonization rate among the participants between the States. In Enugu, secondary school students had significantly higher MRSA colonization rate (42.0%) than primary pupils (18.8%) (p=0.0002), indicating that older students were the more affected educational group in that State. In contrast, a reverse pattern was seen in Ebonyi State, with primary school pupils showing higher but insignificant MRSA colonization rate of 38.1% compared to 27.6% in secondary school students (p=0.1418). These contrasting patterns suggest that younger children in Ebonyi State are the most vulnerable educational group, likely due to poorer personal hygiene practices, close-contact play behavior, overcrowding, and limited ability of school sanitation facilities factors documented to drive MRSA transmission in younger age groups (34). However, in line with the findings from Enugu State, a higher prevalence among age group 10-29 years where secondary school students fall within in another study has also been reported (21). The higher rates among older students in Enugu State may reflect greater mobility, wider social networks, and increased participation in peer-group activities.

Methicillin-resistant *S. aureus* isolates were highly resistant to several commonly used antibiotics in both Enugu and Ebonyi States. Markedly, elevated resistance levels of MRSA isolates were evident for against penicillin (97.9%), sulphamethoxazole (90.7%), erythromycin (76.8%), tetracycline (75.7%), and clindamycin (68.7%), reflecting broad decreased susceptibility to both β-lactam and various non-β-lactam antibiotics. The near universal resistance to penicillin observed in this study agrees with international findings that *S. aureus* exhibits widespread resistance to β-lactam antibiotics due to β-lactamase production and *mecA*-mediated alterations in penicillin-binding proteins. Comparable researches have also reported elevated MRSA resistance to erythromycin, clindamycin, and

tetracycline, indicating the persistent challenge of resistance to these commonly administered antibiotics across various regions, including Africa (14, 33,36,37,38). These trends clearly imply that misuse of antibiotics and unrestricted access to antimicrobial agents in community settings may promote the continued presence and proliferation of resistant MRSA strains.

Contrastingly in this study, MRSA isolates displayed relatively low resistance to gentamicin and ciprofloxacin suggesting these antibiotics may still be partially effective against MRSA isolates in the region. These results are consistent with previous records where *S. aureus* isolates exhibited lower resistance to gentamicin and ciprofloxacin compared to β-lactam antibiotics and tetracycline (35,36,43). Despite this, widespread multidrug-resistance reported in MRSA emphasize the urgent need for sustained antimicrobial surveillance and implementation of antibiotic stewardship programs. Infection control strategies need to be reinforced together with the adoption of evidence-based antibiotic policies is crucial to curb the spread of resistant strains and optimize treatment effectiveness. The MARI further reinforce that Ebonyi State is at higher risk for antimicrobial resistance emergence as more MRSA isolates clustered in the upper MARI categories (0.7–0.8), suggesting higher antibiotic pressure and more sustained transmission environments compared with Enugu.

Conclusion:

In conclusion, the findings from this study highlights the need for state-specific infection control strategies, with priority attention directed towards female pupils and primary school children in Ebonyi, as well as older students in Enugu. Ciprofloxacin and gentamicin may still be effective for the treatment of MRSA infections in this setting.

Contributions of authors:

UEG and OES contributed to the conception and design of the study; UEG coordinated the sample collection and performed the laboratory analysis. UEG and OES participated in the writing of the manuscript, critically reviewed and edited the manuscript. Both authors read and approved the final version of the manuscript.

Source of funding:

Authors received no external funding.

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Taylor, T. A., Tobin, E. H., and Unakal, C. G. *Staphylococcus aureus* infection. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK441868/.
2. Hamwi, A. M., and Salem-Sokhn, E. Methicillin-resistant *Staphylococcus aureus* (MRSA) and antimicrobial resistance in Lebanon: a scoping review. Microbe. 2025; 7: 100405. doi: 10.1016/j.microb.2025.100405.
3. Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., et al. Methicillin-resistant *Staphylococcus aureus:* an overview of basic and clinical research. Nat Rev Microbiol. 2019; 17 (4): 203–218. doi:10.1038/s41579-018-0147-4.
4. Lee, A. S., de Lencastre, H., Garau, J., et al. Methicillin-resistant *Staphylococcus aureus*. Nat Rev Dis Primers. 2018; 4: 18033. doi: 10.1038/nrdp.2018.33.
5. Choo, E. J. Community-associated methicillin-resistant *Staphylococcus aureus* in nosocomial infections. Infect Chemother. 2017; 49 (2): 158–159. doi:10.3947/ic.2017.49.2.158.
6. Tobin, E. H., Jogu, P., and Koirala J. Methicillin-resistant *Staphylococcus aureus.* In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2026. https://www.ncbi.nlm.nih.gov/books/NBK482221/.
7. Pal, M., Rebuma, T., Regassa, T., and Zende, R. Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major threat to public health. Am J Publ Health Res. 2024; 12 (3): 48–53. doi:10.12691/ajphr-12-3-2.
8. Touaitia, R., Mairi, A., Adam, I. N., Suliman, B. N., Takfarinas, I., and Touati, A. *Staphylococcus aureus:* a review of the pathogenesis and virulence mechanisms. Antibiotics. 2025; 14 (5): 470. doi:10.3390/antibiotics14050470.
9. Bashabsheh, R. H. F., Al-Fawares, O., Natsheh, I., Bdeir, R., Al-Khreshieh, R. O., and Bashabsheh, H. H. F. *Staphylococcus aureus* epidemiology, pathophysiology, clinical manifestations and application of nanotherapeutics as a promising approach to combat methicillin-resistant *Staphylococcus aureus.* Pathog Glob Health. 2024; 118 (3): 209–231. doi:10.1080/20477724.2023.2285187.
10. van Tonder, A. J., McCullagh, F., McKeand, H., et al. Colonization and transmission of *Staphylococcus aureus* in schools: a citizen science project. Microb Genom. 2023; 9: 000993. doi:10.1099/mgen.0.000993.
11. Mitevska, E., Wong, B., Surewaard, B. G. J., and Jenne, C. N. The prevalence, risk, and management of methicillin-resistant *Staphylococcus aureus* infection in diverse populations across Canada: a systematic review. Pathogens. 2021; 10 (4): 393. doi:10.3390/pathogens10040393.
12. Sakr, A., Brégeon, F., Mège, J. L., Rolain, J. M., and Blin, O. *Staphylococcus aureus* nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. Front Microbiol. 2018; 9: 2419. doi:10.3389/fmicb.2018.02419.
13. Alshomrani, M. K., Alharbi, A. A., Alshehri, A. A., Arshad, M., and Dolgum, S. Isolation of *Staphylococcus aureus* urinary tract infections at a community-based healthcare center in Riyadh. Cureus. 2023; 15 (2): e35140. doi:10.7759/cureus.35140.
14. Azzam, A., Khaled, H., Fayed, H. M., et al. Prevalence, antibiogram, and risk factors of methicillin-resistant *Staphylococcus aureus* (MRSA) asymptomatic carriage in Africa: a systematic review and meta-analysis. BMC Infect Dis. 2025; 25 (1): 505. doi:10.1186/s12879-025-10819-4.
15. Wangai, F. K., Masika, M. M., Maritim, M. C., and Seaton, R. A. Methicillin-resistant *Staphylococcus aureus* (MRSA) in East Africa: red alert or red herring?

BMC Infect Dis. 2019; 19: 596. doi:10.1186/s12879-019-4245-3.
16. Bilyaminu, U., Saggah, A. M., Abubakar, B. M., and Moi, I. M. Methicillin-resistant *Staphylococcus aureus* colonization and associated factors in clinical isolates among patients attending a tertiary medical centre in Nigeria. BMC Infect Dis. 2025; 25 (1): 1777. doi:10.1186/s12879-025-12166-w.
17. Omosigho, P., Amadin, P., and Okesanya, O. Prevalence of methicillin-resistant *Staphylococcus aureus* in a Nigerian healthcare setting: findings from Edo Specialist Hospital. Microbes Infect Dis. 2024. doi:10.21608/mid.2024.303103.2066.
18. Morufat, A. B., Oluwasegun, O. H., Ibraheem, H. A., Favor, O. I., Christianah, O. B., and Oguntunji, O. Prevalence of methicillin-resistant *Staphylococcus aureus* among students in the College of Health Sciences at Ladoke Akintola University of Technology, Ogbomoso. Sokoto J Med Lab Sci. 2025; 10 (2): 334–345. doi:10.4314/sokjmls.v10i2.36.
19. Campbell, R., Martin, D., Pierce, D., and Nweze, S. Community-associated MRSA among Indigenous children in remote settings: best practices for nurse practitioners. Nurse Pract. 2020; 45 (10): 34–40. doi:10.1097/01.NPR.0000696916.42368.1a.
20. Tuta, K. E., Okesola, A. O., and Umeokonkwo, C. D. The prevalence and risk factors associated with nasal methicillin-resistant *Staphylococcus aureus* colonization among children in a tertiary hospital in Nigeria. Ethiop J Health Sci. 2019;29(4):487–494. doi:10.4314/ejhs.v29i4.10.
21. Said, K. B., Alshammari, K., Ahmed, R. M. E., et al. MRSA profiles reveal age- and gender-specificity in a tertiary care hospital: high burden in ICU elderly and emerging community patterns in youth. Microorganisms. 2025; 13 (5): 1078. doi:10.3390/microorganisms13051078.
22. Albusayes, N. N., Binkhamis, K., Alselaimy, R. M., et al. Prevalence and factors associated with methicillin-resistant *Staphylococcus aureus* colonization among clinical medical students. J Nat Sci Med. 2019; 2 (4): 226. doi:10.4103/JNSM.JNSM_3_19.
23. Oliveira, E. M. N., Carvalho, A. R. B., Ferreira, A. M., et al. Colonization of methicillin-resistant *Staphylococcus aureus* among healthcare students: an integrative review. Sao Paulo Med J. 2021; 139 (6): 607–614. doi:10.1590/1516-3180.2020.0564.R2.22042021.
24. Mason, C. Y., Sobti, A., and Goodman, A. L. *Staphylococcus aureus* bacteriuria: implications and management. JAC Antimicrob Resist. 2023; 5 (1): dlac123. doi:10.1093/jacamr/dlac123.
25. Ehiaghe, J. I., Oshuare, J. R., Amengialue, O. O., and Ayanlere, K. M. Prevalence of *Staphylococcus aureus* and *Escherichia coli* from urine samples of asymptomatic undergraduate students at Benson Idahosa University, Benin City, Nigeria. South Asian J Res Microbiol. 2025; 19 (6): 63–75. doi:10.9734/sajrm/2025/v19i6443.
26. World Gazette. Colourful map of Nigeria with 36 States. 2011. http://www.world.gazette.com.
27. Omar, K., Thabet, H. S., TagEldin, R. A., et al. Ecological niche modeling for predicting the potential geographical distribution of Aedes species (Diptera: Culicidae): a case study of Enugu State, Nigeria. Parasite Epidemiol Contr. 2021; 15: e00225. doi:10.1016/j.parepi.2021.e00225.
28. National Bureau of Statistics. Annual Abstract of Statistics. 2016: 16: 3–12.
29. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310 (20): 2191–2194. doi:10.1001/jama.2013.281053.
30. Cheesbrough, M. District laboratory practice in tropical countries. Part 2. 2nd ed. Cambridge: Cambridge University Press; 2010.
31. Ukpai, E. G., Chukwura, E. I., Moses, I. B., et al. Prevalence and antibiogram of healthcare-associated methicillin-resistant *Staphylococcus aureus* in Ebonyi State, Nigeria. Int J Pharm Sci Rev Res. 2021; 69 (1): 104–111.
32. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI document M100. Wayne: CLSI; 2024.
33. Abdullahi, S., Abdullahi, I., Adekola, H., et al. Nasal carriage rate and multiple antimicrobial resistance indices of *Staphylococcus aureus* among healthcare students at Ahmadu Bello University, Nigeria. Afr J Lab Med. 2025; 14 (1): 1–10. doi:10.4102/ajlm.v14i1.2667.
34. del Rosal, T., Méndez-Echevarría, A., Garcia-Vera, C., et al. *Staphylococcus aureus* nasal colonization in Spanish children: the COSACO nationwide surveillance study. Infect Drug Resist. 2020; 13: 4643–4651. doi:10.2147/IDR.S282880.
35. Ogefere, H. O., Umaru, G., Ibadin, E. E., and Omoregie, R. Prevalence of methicillin-resistant staphylococci among apparently healthy students attending a tertiary institution in Benin City, Nigeria. Niger J Basic Appl Sci. 2019; 27 (1): 114–121. doi:10.4314/njbas.v27i1.15.
36. Akerele, J., Obasuyi, O., and Omede, D. Prevalence of methicillin-resistant *Staphylococcus aureus* among healthy residents of Ekosodin community in Benin City, Nigeria. Trop J Pharm Res. 2015; 14: 1495–1501. doi:10.4314/tjpr.v14i8.24.
37. Okwu, M. U., Akpoka, A. O., Mitsan, O., Izevbuwa, O. E., Osamede, A., and Tkadlec, J. High frequency of methicillin-resistant and multidrug-resistant strains of *Staphylococcus aureus* colonizing students in Okada, Edo State, Nigeria. Microb Drug Resist. 2023; 29 (11): 516–522. doi:10.1089/mdr.2023.0001.
38. Azzam, A., Khaled, H., Salem, A., et al. Proportion and antibiogram of methicillin-resistant *Staphylococcus aureus* (MRSA) in Africa: a systematic review and meta-analysis. Antimicrob Resist Infect Contr. 2026; 15 (1): 13. doi:10.1186/s13756-025-01687-3.
39. Dembski, S. C., Giedroyc, C., Karol, N., Misra, T., and Guthrie, J. L. Social determinants and community-level risk factors in CA-MRSA transmission among disadvantaged populations in North America: a scoping review. Epidemiol Infect. 2025; 153: e126. doi:10.1017/S0950268825100691.
40. Loewen, K., Schreiber, Y., Kirlew, M., Bocking, N., and Kelly, L. Community-associated methicillin-resistant *Staphylococcus aureus* infection: literature review and clinical update. Can Fam Physician. 2017; 63 (7): 512–520.
41. Yadav, K., and Prakash, S. Prevalence and antibiotic susceptibility pattern of MRSA in upper respiratory tract infection. Glob J Microbiol Res. 2016; 4 (2): 167–173.
42. Kodde, C., Bonsignore, M., Köhler, J., Schwegmann, K., and Nachtigall, I. Males are at higher risk of colonization and infection with multidrug-resistant organisms than females. J Hosp Infect. 2025; 155: 88–94. doi:10.1016/j.jhin.2024.07.021.
43. Ahmadi, E., Khojasteh, M., Mortazavi, S., et al. Prevalence of and risk factors for methicillin-resistant *Staphylococcus aureus* nasal carriage in the west of Iran: a population-based cross-sectional study. BMC Infect Dis. 2019; 19: 899. doi:10.1186/s12879-019-4567-1.

Jugah et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 309 - 319 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2496. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Prevalence of New Delhi metallo-β-lactamase gene among multi-drug-resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates at the University College Hospital, Ibadan, Nigeria

*[1,2]Jugah, C. C., [1,3]Makanjuola, O. B., [1]Ogunleye, V. O., [4]Azuh, V. O., [4]Kazeem, O. S., [1,4]Subulade, A. A., [4]Adesanya, O. A., [4]Adeniyi, A. V, and [1,3]Kehinde, A. O.

[1]Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Nigeria
[2]College of Medicine, University of Ibadan, Nigeria
[3]University College Hospital, Ibadan, Nigeria
[4]Biorepository and Clinical Virology Laboratory, College of Medicine, University of Ibadan, Nigeria
*Correspondence to: christianaokiyi25@gmail.com

Abstract:

Background: Failure of antimicrobial therapy may give an insight into carriage of resistance genes among microbial pathogens. The ease of spread of these genes including beta-lactamase genes such as *bla*$_{NDM}$, *bla*$_{KPC, and}$ *bla*$_{OXA-48}$ is a major public health challenge worldwide. The study aimed to detect New Delhi metallo-β-lactamase (*bla*$_{NDM}$) gene carriage among multidrug-resistant (MDR) *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates from clinical specimens at the University College Hospital, Ibadan (UCH), Ibadan, Nigeria.
Methodology: This was a laboratory-based study of 110 presumptively identified Gram-negative bacterial isolates at the routine microbiology laboratory of UCH Ibadan, Nigeria between May and August 2021. The isolates were re-identified and confirmed as *E. coli*, *K. pneumoniae* and *P. aeruginosa* using biochemical tests, including oxidase test, cetrimide agar test, and Analytical Profile Index (API) system. Antibiotic susceptibility test of confirmed isolates to selected antibiotics was done using the modified Kirby-Bauer disk diffusion method and multi-drug resistance was defined as resistance to 3 or more antibiotic classes. The *bla*$_{NDM}$ was detected among the MDR isolates by conventional polymerase chain reaction using specific primers. Descriptive statistics were used to summarize variables, and Chi-square was used to compare categorical variables, with p value ≤ 0.05 taken to be statistically significant.
Results: Of the total 110 Gram-negative bacilli collected from the routine microbiology laboratory, 90 were confirmed as the Gram-negative bacilli of interest, with *K. pneumoniae* (58.9%, n=53), *E. coli* (31.1%, n=28) and *P. aeruginosa* (10.0%, n=9). Majority of the isolates (n=77, 85.6%) were susceptible to ertapenem, while most were resistant to ciprofloxacin (n=75, 83.3%), and 64 (71.1%) were multidrug-resistant. Among the MDR isolates, NDM carriage rate was 12.5% (n=8/64), with the carriage rate highest in *P. aeruginosa* (50.0%, n=1/2), followed by *K. pneumoniae* (13.6%, n=6/44) and *E. coli* (5.6%, n=1/18) but no significant difference in the carriage rate (x^2=3.417, p=0.1811).
Conclusion: This study shows a high prevalence of MDR Gram-negative bacilli, with *K. pneumoniae* being the highest. Resistance was mostly observed to ciprofloxacin, while most isolates were susceptible to ertapenem. *bla*$_{NDM}$ gene was detected in all the three MDR bacterial pathogens. Emphasis should be placed on rational antibiotic use and routine susceptibility testing, including resistance gene detection, to guide therapy, and prevent emergence of multidrug resistance in microbial population.

Keywords: Multi-drug resistance; New Delhi metallo-β-lactamase; Carriage; Gram-negative bacilli

Received Feb 12, 2026; Revised Apr 13, 2026; Accepted Apr 14, 2026

Prévalence du gène de la métallo-β-lactamase New Delhi parmi les isolats cliniques d'*Escherichia coli*, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa* multirésistants à l'Hôpital Universitaire d'Ibadan, au Nigéria

*[1,2]Jugah, C. C., [1,3]Makanjuola, O. B., [1]Ogunleye, V. O., [4]Azuh, V. O., [4]Kazeem, O. S., [1,4]Subulade, A. A., [4]Adesanya, O. A., [4]Adeniyi, A. V., et [1,3]Kehinde, A. O.

[1]Département de Microbiologie Médicale et de Parasitologie, Collège de Médecine, Université d'Ibadan, Nigéria
[2]Collège de Médecine, Université d'Ibadan, Nigéria
[3]Hôpital Universitaire d'Ibadan, Nigéria
[4]Biobanque et Laboratoire de Virologie Clinique, Collège de Médecine, Université d'Ibadan, Nigéria Ibadan, Nigéria
*Correspondance à: christianaokiyi25@gmail.com

Résumé:

Contexte: L'échec d'un traitement antimicrobien peut révéler la présence de gènes de résistance chez les agents pathogènes microbiens. La facilité de propagation de ces gènes, notamment les gènes de bêta-lactamases tels que *bla*$_{NDM}$, *bla*$_{KPC}$ et *bla*$_{OXA-48}$, constitue un enjeu majeur de santé publique à l'échelle mondiale. Cette étude visait à détecter la présence du gène de la métallo-β-lactamase de New Delhi (*bla*$_{NDM}$) chez des isolats d'*Escherichia coli*, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa* multirésistants (MDR) provenant d'échantillons cliniques prélevés au Centre Hospitalier Universitaire d'Ibadan (UCH), à Ibadan, au Nigéria.
Méthodologie: Cette étude de laboratoire a porté sur 110 isolats bactériens à Gram négatif présumés, identifiés au laboratoire de microbiologie de routine du CHU d'Ibadan, au Nigéria, entre mai et août 2021. Ces isolats ont été réidentifiés et confirmés comme étant des espèces d'*E. coli*, *K. pneumoniae* et *P. aeruginosa* par des tests biochimiques, notamment le test de l'oxydase, le test sur gélose au cétrimide et le système API (Index du Profil Analytique). La sensibilité aux antibiotiques des isolats confirmés a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer modifiée. La multirésistance a été définie comme une résistance à au moins trois classes d'antibiotiques. Le gène *bla*$_{NDM}$ a été recherché parmi les isolats multirésistants par PCR conventionnelle à l'aide d'amorces spécifiques. Des statistiques descriptives ont été utilisées pour résumer les variables, et le test du χ^2 a permis de comparer les variables catégorielles. Un seuil de signification statistique de $p \leq 0,05$ a été retenu.
Résultats: Sur un total de 110 bacilles Gram négatifs collectés au laboratoire de microbiologie de routine, 90 ont été confirmés comme étant les bacilles Gram négatifs d'intérêt, avec *K. pneumoniae* (58,9%, n=53), *E. coli* (31,1%, n=28) et *P. aeruginosa* (10,0%, n=9). La majorité des isolats (n=77, 85,6%) étaient sensibles à l'ertapénem, tandis que la plupart étaient résistants à la ciprofloxacine (n=75, 83,3%) et 64 (71,1%) étaient multirésistants. Parmi les isolats multirésistants (MDR), le taux de portage de NDM était de 12,5% (n=8/64), avec le taux le plus élevé chez *P. aeruginosa* (50,0%, n=1/2), suivi de *K. pneumoniae* (13,6%, n=6/44) et d'*E. coli* (5,6%, n=1/18). Cependant, aucune différence significative n'a été observée entre les taux de portage (χ^2=3,417, p=0,1811).
Conclusion: Cette étude révèle une forte prévalence de bacilles Gram négatifs multirésistants, *K. pneumoniae* présentant le taux le plus élevé. La résistance était principalement observée à la ciprofloxacine, tandis que la plupart des isolats étaient sensibles à l'ertapénem. Le gène *bla*$_{NDM}$ a été détecté chez les trois bactéries pathogènes multirésistantes. Il convient de privilégier un usage rationnel des antibiotiques et la réalisation systématique de tests de sensibilité, incluant la détection des gènes de résistance, afin d'orienter le traitement et de prévenir l'émergence de la multirésistance au sein des populations microbiennes.

Mots-clés: Multirésistance; Métallo-β-lactamase de New Delhi; Portage; Bacilles Gram négatifs

Introduction:

Infections caused by multidrug resistant (MDR) organisms result in prolonged hospital stays, increased healthcare costs, and increased mortality and morbidity due to limited treatment options (1). The increasing prevalence of MDR pathogens has become a major public health challenge worldwide (2,3). In Nigeria, studies have reported high prevalence of 70-90% multidrug resistance among clinical isolates (4,5). Several factors have contributed to the increasing trend in microbial drug resistance. The ease of spread of resistance genes is a major challenge coupled with their enhanced selection as a result of drug misuse and abuse by patients and individuals (6).

Over-the-counter availability of antibiotics, lack of surveillance and routine susceptibility testing in resource-limited countries have contributed immensely to irrational antimicrobial drug usage, thus compounding the present challenge of antimicrobial resistance (AMR) (7, 8). Other factors such as overuse of antibiotics for treatment and growth promotion in animals have been noted to contribute to the development and spread of resistance (9,10). The limited scientific strategies to develop new classes of antibiotics have also partly contributed to the proliferation of resistance in MDR organisms (11).

Resistance of microbes to antimicrobial agents is attributed to their ability to produce enzymes such as extended-spectrum beta-lactamase (ESBL) that inhibit the effect of these agents (12). Of great concern are MDR organisms resistant to the carbapenems, which are considered the drugs of 'last resort' for treating infections caused by these pathogens (13). Organisms resistant to carbapenems have been isolated from various sources, including human and animal populations and the environment

(14). Outbreaks caused by carbapenemase-producing Enterobacteriaceae have also been reported from patient samples (15).

The horizontal transmission of carbapenemase genes, mediated by mobile genetic elements carrying additional resistance elements, confers resistance to various groups of antibiotics, resulting in MDR (16,17). Carbapenemases belong to molecular class A (KPC), class B (MBL including NDM, IMP and VIM) and class D (OXA-48-like carbapenemase) according to the Ambler classification system (18,19). The production of carbapenemase has been shown to be the main mechanism of carbapenem resistance in epidemiological studies (20,21). MDR isolates categorized as being susceptible to carbapenem by phenotypic tests can still carry carbapenemase resistance gene on molecular testing (22).

The occurrence of multiple resistance determinants including more than two carbapenemases in one isolate has also been frequently reported (23,24). Decreased permeability, ESBL production and efflux pump over-expression are noted to play a role in carbapenem resistance (25,26). Antibiotics such as ceftazidime/avibactam, aztreonam/ avibactam, colistin, tigecycline and fosfomycin have been recommended for the treatment of infections caused by carbapenem-resistant Enterobacterales (CRE) (27).

Two-thirds of all healthcare associated infections have been reported to originate from just six MDR organisms, including *Enterobacter* species, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, *Staphylococcus aureus* (methicillin-resistant *S. aureus* -MRSA, vancomycin-resistant *S. aureus*-VRSA) and *Enterococcus* species (vancomycin resistant enterococci-VRE). These organisms are collectively known as the ESKAPE pathogens due to their ability to evade the mechanism of action of several antibiotics (28). *Escherichia coli*, *Klebsiella* spp and *P. aeruginosa*, identified as priority pathogens by the World Health Organization (WHO), are among the most common pathogens responsible for nosocomial infections, and their emerging resistance to a wide range of antibiotics poses a major challenge to patient treatment and public health at large (29,30).

Although there are extensive records on the phenotypic resistance patterns of these organisms, very little is known about the genes responsible for resistance in most MDR isolates in Nigeria (31). Local studies have reported susceptibility profiles of many of these organisms against antimicrobial agents without indicating the resistance genes responsible (32). Despite the existence of MDR among enteric bacteria in Nigeria, there is limited information regarding the genes responsible for resistance (33,34). This study therefore aimed to detect the presence of NDM resistance gene in three common MDR Gram-negative bacilli of medical importance (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*).

Materials and method:

Study design and site:

This laboratory-based study of selected Gram-negative bacterial isolates (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) was carried out at the University College Hospital (UCH), Ibadan, from May to August 2021. UCH is a major tertiary healthcare and referral centre in south-western Nigeria, providing specialized medical services and serving as a training and research institution for healthcare professionals.

Ethical approval:

The study was approved by the Ethics and Research Committee (UI/EC/21/0179) of the University College Hospital and University of Ibadan, Nigeria.

Source of bacterial isolates:

The bacterial isolates (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) presumptively identified from clinical samples (urine, sputum, wound swabs, biopsy materials, body fluids, and blood cultures) submitted for routine diagnostic testing, were obtained by consecutive sampling from the Medical Microbiology and Parasitology Laboratory in UCH, Ibadan.

Re-identification and phenotypic confirmation of selected bacterial isolates:

A total of 110 collected Gram-negative bacterial isolates were re-characterised to confirm their identity. Gram staining technique was done for confirmation of Gram-negative bacilli. Identification of species of the Gram-negative bacilli was carried out using oxidase test, cetrimide agar test, and the Analytical Profile Index (API) system.

Oxidase test:

The oxidase test was carried out using impregnated oxidase strips. A sterile loop was used to pick a pure inoculum and smeared on the strip. A purple colour change observed within 10 seconds was recorded as positive (36). This test was used to differentiate oxidase-positive bacteria such as *Pseudomonas* spp from oxidase-negative bacteria, including *E. coli* and *Klebsiella* spp, among the selected bacterial isolates.

Cetrimide agar test:

Cetrimide agar (Oxoid, UK) was used to selectively isolate and identify *P. aeruginosa*. A

loopful of the oxidase positive isolate was streaked on the surface of the agar and incubated at 37 °C for 24–48 hours. The presence of greenish pigment (pyocyanin) and characteristic grape-like odor was considered indicative for *P. aeruginosa* growth (37).

Analytical profile index (API) system (API-20E):

The species of the presumptive fermentative Gram-negative bacteria isolates were identified using the API-20E test. A suspension of each pure isolate from 24-hour growth was prepared using normal saline and adjusted to 0.5 MacFarland standard. The bacterial suspension was used to rehydrate each of the wells on the API strip, which was then incubated for 24 hours. Colour changes were observed after incubation following manufacturer's instructions.

The positive and negative results were compiled to obtain a profile number, which was interpreted online to determine the identity of the isolate (38). This test was used to confirm the identity of members of the family Enterobacteriaceae, including *E. coli* and *Klebsiella* spp among the selected bacterial isolates.

Antibiotic susceptibility testing of the selected isolates:

The antibiotic susceptibility of the confirmed isolates was tested on Muller-Hinton agar using the modified Kirby-Bauer disc diffusion method. Seven antibiotics (Oxoid, UK); amoxicillin-clavulanic acid (30µg), gentamicin (10µg), ciprofloxacin (5µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), aztreonam (30 µg) and ertapenem (10µg) were tested. Inoculum of each confirmed isolate was prepared and standardized to 0.5 MacFarland (~1.5 x 10^8 CFU /ml) and then inoculated on Mueller-Hinton agar plates. Antibiotic disc**s** were applied, and plates were incubated at 37ºC overnight. The inhibition zone diameters were measured and interpreted as susceptible or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines (39). *Escherichia coli* ATCC 25922 was used as control strain.

Isolates resistant to at least three antibiotics classes were regarded as multidrug resistant. MDR isolates were sub-cultured into Tryptic Soy Broth (TSB) and then stored at -20 ºC to ensure preservation of viable cells for molecular analysis (36).

Resistance gene detection by PCR:

The stored isolates were retrieved for molecular analysis, which was carried out in the Biorepository and Clinical Virology Laboratory, College of Medicine, UCH, Ibadan.

DNA extraction by chemical method:

Bacterial broth from pure culture was transferred into 2ml microtubes and centrifuged at 10,000 rpm for 5 minutes. Pellets were re-suspended in 520 µL of Tris-EDTA (TE) buffer and vortexed. Fifteen microliters of 20% sodium dodecyl sulphate (SDS) were added, and the mixture was incubated at 37ºC for 1 hour. A solution of 100µL of 5 M NaCl and 80 µL of 10% cetyltrimethylammonium bromide (CTAB) in 0.7 M NaCl was added and vortexed, followed by incubation at 65ºC for 10 minutes and cooling on ice for 15 minutes. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, vortexed, incubated on ice for 5 minutes, and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred to a new tube, and isopropanol (1:0:6) was added to precipitate DNA, followed by incubation at -20ºC for 1 hour.

The DNA was collected by centrifugation at 12,000 rpm for 10 minutes, washed with 500 µL of 70% ethanol, centrifuged for 5 minutes at 10,000 rpm, air-dried at room temperature for 3 hours, and dissolved in 50 µL of distilled water (35)

Amplification by conventional PCR:

The NDM gene was detected by PCR assay using specific primer targeting bla_{NDM} in the MDR isolates. Specific primers for PCR (NDM forward: CCAATATTATGCACCCGGTCG; reverse: ATGCGGGCCGTATGAGTGATTG) used have been previously documented by Handal et al., (40). Reaction cocktail used for the PCR primer set included 5 x PCR SYBR Green buffer (2.5µl), $MgCl_2$ (0.75µl), 10pM dNTP (0.25µl), 10pM each forward and reverse primers (0.25µl), 8000 U of Taq DNA polymerase (0.06µl) which was made up to 10.5µl with sterile distilled water to which 2µl template was added. The primer mix, master mix, and water were added to the well plate, after which the DNA template was added to the plate.

The PCR conditions include initial denaturation at 94ºC for 5 minutes, followed by 30 cycles consisting of denaturation at 95ºC for 30 seconds, annealing at 51ºC for 40 seconds, extension at 72ºC for 40 seconds and final extension of 72ºC for 5 minutes. The PCR set up was allowed to run for at least 1 hour 45 minutes for completion of the PCR. The PCR was carried out in GeneAmp 9700 PCR system thermal cycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for the primer pair.

Gel electrophoresis of amplified products:

The PCR amplicons were resolved on

2% agarose gel prepared in Tris-acetate-EDTA (TAE) buffer. Electrophoresis was carried out at a constant voltage for approximately 1 hour. DNA bands were visualized under ultraviolet light and compared with a molecular weight marker (DNA ladder). Bands corresponding to the expected size of the target gene, as observed in the positive control, were recorded as positive, while absence of such bands was recorded as negative. A *Klebsiella pneumoniae* isolate harboring the bla_{NDM-1} gene was used as the positive control.

Statistical analysis:

Data were sorted, coded, and cleaned. Descriptive statistics (percentages) were used to summarize variables. Chi-square test was used to assess the association between bacterial species (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) and multidrug resistance, as well as the relationship between bacterial species and NDM gene carriage. A p value ≤ 0.05 was considered significant. Analysis was performed using SPSS version 26.0.

Results:

Frequency distribution of the selected bacterial isolates:

Of a total of 110 Gram-negative isolates collected from the routine laboratory, 90 were biochemically confirmed, with *K. pneumoniae* (n= 53; 58.9%), *E. coli* (n=28; 31.1%), and *P. aeruginosa* (n=9; 10.0%) (Fig 1). The highest frequency of Gram-negative isolates was from urine samples (n=25, 27.8%), followed by wound swab/biopsy (n=23, 25.6%), sputum (n=13, 14.4%), and blood culture (n=12, 13.3%). *Klebsiella pneumoniae* (n=13, 24.5%) and *E. coli* (n=11, 39.3%) were most frequently isolated from urine, while *P. aeruginosa* (n=3, 33.3%) was predominantly obtained from ear swabs (Table 1).

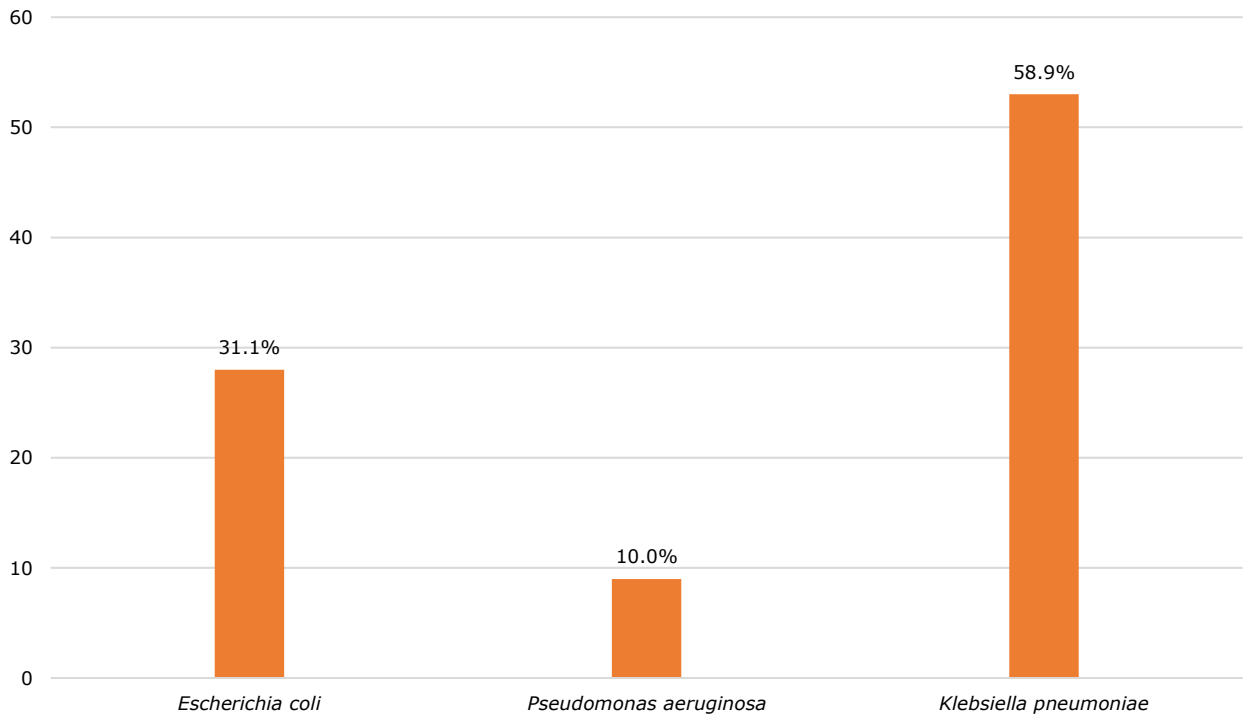


Fig 1: Frequency distribution of selected Gram-negative clinical bacterial isolates

Table 1: Distribution of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates according to clinical samples

Sample	***Escherichia coli* (%)**	***Klebsiella pneumoniae* (%)**	***Pseudomonas aeruginosa* (%)**	**Total (%)**
Sputum	1 (3.6)	11 (20.8)	1 (11.1)	13 (14.4)
Wound swab/biopsy	9 (32.1)	12 (22.6)	2 (22.2)	23 (25.6)
Tracheal aspirate	1 (3.6)	2 (3.8)	1 (11.1)	4 (4.4)
Blood culture	4 (14.3)	9 (17.0)	0	13 (14.4)
Ear swab	0	2 (3.8)	3 (33.3)	5 (5.6)
Intracervical fluid	0	1 (1.9)	0	1 (1.1)
Pleural biopsy/fluid	1 (3.6)	1 (1.9)	1 (11.1)	3 (3.3)
Pus	0	1 (1.9)	0	1 (1.1)
Tissue biopsy	1 (3.6)	1 (1.9)	0	2 (2.2)
Urine	11 (39.3)	13 (24.5)	1 (11.1)	25 (27.8)
Total	**28 (31.1)**	**53 (58.9)**	**9 (10.0)**	**90 (100.0)**

Susceptibility patterns of isolates to antibiotics:

Table 2 presents the antibiotic susceptibility of the bacterial isolates. Among *E. coli* isolates, resistance was highest to ciprofloxacin (n=25/28, 89.3%), followed by aztreonam (n= 18/28, 64.3%) and amoxicillin-clavulanic acid (n=17/28; 60.7%).

Most of the *K. pneumoniae* isolates were resistant to ciprofloxacin (n=47/53, 88.7%), ceftazidime (n=42/53; 79.3%), aztreonam (n=39/ 53, 73.6%) and gentamicin (n=39/53; 73.6%). In contrast, *P. aeruginosa* isolates showed higher susceptibility to most antibiotics tested compared to *E. coli* and *K. pneumoniae*.

Most of the isolates tested were susceptible to ertapenem with *E. coli* (n=27, 96.4%), *K. pneumoniae* (n=43, 81.3%) and *P. aeruginosa* (n=7, 77.8%).

Table 2: Antibiotic susceptibility of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Antimicrobial category (Antibiotics)	***Escherichia coli* (n=28)**			***Pseudomonas aeruginosa* (n=9)**			***Klebsiella pneumoniae* (n=53)**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Aminoglycoside (Gentamicin)	14 (50)	0	14 (50)	6 (66.7)	0	3 (33.3)	13 (24.5)	1 (1.9)	39 (73.6)
Fluoroquinolone (Ciprofloxacin)	1 (3.6)	2 (7.1)	25 (89.3)	6 (66.7)	0	3 (33.3)	4 (7.6)	2 (3.8)	47 (88.7)
Monobactam (Aztreonam)	4 (14.3)	6 (21.4)	18 (64.3)	6 (66.7)	2 (22.2)	1 (11.1)	11 (20.8)	4 (7.6)	39 (73.6)
Third generation cephalosporin (Ceftazidime)	8 (28.6)	5 (17.9)	15 (53.6)	7 (77.8)	0	2 (22.2)	7 (13.2)	4 (7.6)	42 (79.3)
Penicillin + β-lactamase inhibitors (Amoxicillin-clavulanic acid) (Piperacillin-tazobactam)	5 (17.8) -	6 (21.4) -	17 (60.7) -	- 5 (55.6)	- 2 (2.2)	- 2 (22.2)	9 (17) -	10 (18.9) -	34 (64.2) -
Carbapenem (Ertapenem)	27 (96.4)	0	1 (3.6)	7 (77.8)	0	2 (22.2)	43 (81.1)	3 (5.7)	7 (13.2)

S=sensitive; I=Intermediate; R=Resistant

Multidrug resistant pattern of the isolates:

Of the 90 Gram-negative bacilli tested, 64 (71.1%) were multidrug resistant (MDR), with resistance to at least three classes of antibiotics. The prevalence of MDR was highest in *K. pneumoniae* (44/53; 83.0%), followed by *E. coli* (18/28; 64.3%), and *P. aeruginosa* (2/9; 22.2%) (x^2=14.764, p=0.0006) (Table 3).

Resistance genes in clinical isolates:

Fig 2 shows the representative image of the NDM gene detected among the MDR clinical isolates on agarose gel electrophoresis with a band size of 1500bp. Positive samples were run alongside a positive control and a 100 bp molecular weight marker (MK).

Of the 64 MDR isolates, 8 (12.5%) harbored the NDM gene; 6 (9.4%) *K. pneumoniae* and 1 (1.6%) each of *P. aeruginosa* and *E. coli* (Table 4).

Table 3: Multidrug resistance among *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Isolates	Number of isolates	Number of MDR isolates	Percentage of MDR isolates	X^2	*p* value
Escherichia coli	28	18	64.3	14.764	0.0006*
Pseudomonas aeruginosa	9	2	22.2		
Klebsiella pneumoniae	53	44	83.0		
Total	**90**	**64**	**71.1**		

*=statistically significant

MK= Marker; = Positive samples

Fig 2: Representative agarose gel electropherogram showing PCR amplified NDM gene products

Table 4: Carriage of NDM gene among multidrug *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Bacterial isolate	Number of MDR isolates	Number of MDR isolates with NDM gene (%)	x^2	*p* value
Escherichia coli (n=28)	18	1 (5.6)	3.417	0.1811
Pseudomonas aeruginosa (n=9)	2	1 (50.0)		
Klebsiella pneumoniae (n=53)	44	6 (13.6)		
Total (n=90)	**64**	**8 (12.5)**		

Discussion:

The identification of the AMR patterns of bacterial isolates plays an important role in guiding rational prescription of antibiotics and management of bacterial infections. The reported organisms in this study have similarities with other studies that have been conducted on MDR organisms. From this study, *K. pneumoniae* was the most predominant, followed by *E. coli*, and *P. aeruginosa*, which agrees with the findings of Mushi et al., (41) and Aly and Balkhy (42), that reported these three bacteria as the prevalent MDR organisms. The 44.0% prevalence of *E. coli* reported by Aly and Balkhy (42) is comparable with the trend observed in the study, while their reported prevalence rates of 20.0% for *K. pneumoniae* and 18.7% for *P. aeruginosa* also support the dominance of these organisms. Other studies likewise reported *P. aeruginosa*, *K. pneumoniae*, *E. coli* and *Enterobacter* species as frequently isolated MDR organisms. The consistent isolation of these organisms across different studies confirm their importance in multidrug-resistant infections (41-44).

The predominance of *K. pneumoniae* and *E. coli* in urine samples in this study is similar to findings reported in other studies (41). Although some studies reported blood as the commonest site of infection, variations in infection sites may be influenced by differences in study settings and geographical locations (44, 45). The widespread occurrence of MDR organisms across different clinical samples further highlights the growing challenge of antimicrobial resistance in healthcare settings (46,47).

From this study, both *E. coli* and *K. pneumoniae* were resistant to ciprofloxacin, which agrees with the report of Gurung et al., (48). *Klebsiella pneumoniae* showed high resistance to ciprofloxacin, ceftazidime, aztreonam and gentamicin, which is similar to the findings of Chen et al., (49), where *K. pneumoniae* was reported to show high resistance to ciprofloxacin, ceftazidime, aztreonam, ertapenem and gentamicin among others. Ertapenem was selected as the antibiotic of choice for testing carbapenem resistance because it is the most sensitive carbapenem indicator for detecting the production of carbapenemase in Enterobacteriaceae. Unlike other carbapenems such as imipenem and meropenem, ertapenem has reduced affinity for AmpC beta-lactamases and porin mutations, which makes it suitable for differentiating carbapenemase-mediated resistance (50).

Over 70% of the isolates were multidrug resistant, indicating a high prevalence of MDR among the organisms investigated. This prevalence is comparable to the 69.6% MDR rate reported by Adenipekun et al., (5), but higher than the 42.1% reported among Enterobacteriaceae isolates by Bitew and Tsige (51), and lower than the 87.4% MDR rate reported by Eshetie et al., (52). The variation in MDR prevalence across studies may be influenced by differences in study populations, sample size, antimicrobial usage patterns, infection control practices, and geographical settings.

Klebsiella pneumoniae accounted for the largest proportion of MDR isolates in this study, constituting 68.8% of the total MDR isolates, indicating its major contribution to multidrug resistance. Furthermore, 83.0% of *K. pneumoniae* isolates were MDR, which was significantly higher than 64.3% observed in *E. coli* and 22.2% in *P. aeruginosa*. This finding supports reports that have identified *K. pneumoniae* as a predominant MDR organism, followed by *E. coli* (48). Multidrug resistance has been attributed to the accumulation and expression of multiple resistance genes on R plasmids or increased expression of genes encoding multidrug efflux pumps, which may result in complex MDR phenotypes and sometimes pan-resistance (48,53). Other studies have also reported MDR *E. coli* from drinking water sources and MDR *Pseudomonas* isolates, further confirming the widespread nature of multidrug resistance among Gram-negative bacteria (54,55).

In this study, 8 of the 64 MDR isolates (12.5%) were positive for the NDM gene, indicating the presence of potential carbapenemase-producing strains among the MDR isolates. This finding is of clinical importance because the NDM gene confers resistance to carbapenems, which are considered drugs of 'last resort' for the treatment of severe Gram-negative infections (13). The detection of NDM among MDR isolates suggests limited therapeutic options and highlights the risk of treatment failure and rapid dissemination of resistance within healthcare settings. The detection of the NDM gene among multidrug-resistant isolates in this study highlights the presence of carbapenem resistance across the studied organisms. However, the NDM carriage rate was highest in *P. aeruginosa* (50.0%), followed by *K. pneumoniae* (13.6%) and *E. coli* (5.6%), although this difference was not statistically significant.

The overall prevalence of NDM observed in this study is comparable to previous reports, including the 10.2% carbapenemase resistance reported by Mohammed et al. and the 14.3% NDM prevalence reported by Tsai et al., (56,57). However, higher NDM prevalence of about 25% has been reported in studies from Kano and Yenagoa, Nigeria, which may be attributed to differences in antibiotic usage patterns, infec-

tion control practices, and study populations (58,59). These findings suggest that NDM-mediated resistance is present across multiple Gram negative organisms rather than being restricted to a single predominant species. The high resistance trend is indicative of high antibiotic selection pressure largely due to relatively cheap cost and easy availability of these agents, mostly used as first line or common choice of treatment in many healthcare settings in these regions (43,44).

Conclusion:

This study showed a high level of multidrug resistance, including the emergence of carbapenem resistance among clinical isolates, as reported in other studies. The highest prevalence of MDR was observed among *K. pneumoniae* isolates. Resistance was mostly to ciprofloxacin, while ertapenem was the antibiotic to which these bacteria were most susceptible.

The NDM gene was detected in *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, with no statistically significant difference in carriage rates among the bacteria. Emphasis should be placed on rational antibiotic use and routine susceptibility testing, including resistance gene detection and sequencing, to guide treatment and curb multidrug resistance.

Acknowledgements:

The authors acknowledge the staff of the Medical Microbiology and Parasitology Laboratory, University College Hospital (UCH), Ibadan, particularly the Medical Laboratory Scientists, for their support and for providing the clinical isolates used in this study.

Contributions of authors:

JCC and KAO were involved in the study conceptualization and design; JCC, OVO and KOS carried out data collection and methodological activities; JCC, AOA and AVO performed the molecular work; AAV conducted the data analysis; JCC and SAA produced the initial draft; and KAO and MOB critically reviewed the initial draft. All authors were involved in findings interpretation, critical review and approval of the final draft.

Source of funding:

No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Serra-Burriel, M, Keys, M, Campillo-Artero, C, et al. Impact of multi-drug-resistant bacteria on economic and clinical outcomes of healthcare-associated infections in adults: Systematic review and meta-analysis. PLoS One. 2020; 15 (1): e0227139. https://doi.org/10.1371/journal.pone.0227139
2. Tacconelli, E, Carrara, E, Savoldi, A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018; 18 (3): 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3.
3. Pitout, J. D., and Laupland, K. B. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008; 8 (3): 159–166. https://doi.org/10.1016/S1473-3099(08)70041-0.
4. Adeyemi, K., Okunrounmu, P., Olagbende, A., Adedokun, O., Hassan, A., and Atilola, G. High prevalence of multiple drug-resistant enteric bacteria: evidence from a teaching hospital in Southwest Nigeria. J Infect Publ Health. 2020; 13 (4): 651–656. https://doi.org/10.1016/j.jiph.2019.08.014.
5. Adenipekun, E. O., Jackson, C. R., Ramadan, H., et al. Prevalence and multidrug resistance of *Escherichia coli* from community-acquired infections in Lagos, Nigeria. J Infect Dev Ctries. 2016; 10 (9): 920-931. https://doi.org/10.3855/jidc.7997.
6. Tenover, F. C. Development and spread of bacterial resistance to antimicrobial agents: an overview. Clin Infect Dis. 2001; 33 Suppl 3: S108–S115. https://doi.org/10.1086/321834.
7. Okeke, I. N. Diagnostic insufficiency in Africa. Clin Infect Dis. 2006; 42 (10): 1501–1503. https://doi.org/10.1086/503308.
8. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015; 40 (4): 277–283.
9. Finley, R. L., Collignon, P., Joakim Larsson, D. G., et al. The scourge of antibiotic resistance: the important role of the environment. Clin Infect Dis. 2013; 57: 704–710. https://doi.org/10.1093/cid/cit355.
10. Tang, Q., Song, P., Li, J., Kong, F., Sun, L., and Xu, L. Control of antibiotic resistance in China must not be delayed: the current state of resistance and policy suggestions for the government, medical facilities, and patients. Biosci Trends. 2016; 10: 1–6. https://doi.org/10.5582/bst.2016.01034.
11. Gupta, V., and Datta, P. Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. Indian J Med Res. 2019; 149 (2): 97–106. https://doi.org/10.4103/ijmr.IJMR_755_18.
12. Reygaert, W. C. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 2018; 4 (3): 482–501. https://doi.org/10.3934/microbiol.2018.3.482.
13. Tam, V. H., Chang, K. T., Abdelraouf, K., et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2010; 54 (3): 1160-1164. https://doi.org/10.1128/AAC.01446-09.
14. Woodford, N., Tierno, P. M Jr., Young, K., et al. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3. Antimicrob Agents Chemother. 2004; 48 (12): 4793–4799. https://doi.org/10.1128/AAC.48.12.4793-4799.2004.
15. Lepuschitz, S., Baron, S., Larvor, E., et al. Phenotypic and genotypic antimicrobial resistance traits of *Vibrio cholerae* non-O1/non-O139 isolated

from a large Austrian lake. Front Microbiol. 2019; 10: 2600. https://doi.org/10.3389/fmicb.2019.02600.
16. Hirsch, E. B., and Tam, V. H. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother. 2010; 65 (6): 1119–1125. doi:10.1093/jac/dkq108
17. Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010; 10 (9): 597–602. https://doi:10.1016/S1473-3099(10)70143-2.
18. Queenan, A. M., and Bush, K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007; 20: 440–458. https://doi:10.1128/CMR.00001-07.
19. van Duin, D., and Doi, Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence, 2017; 8 (4): 460-469. https://doi:10.1080/21505594.2016.1222343.
20. Munoz-Price, L. S., Poirel, L., Bonomo, R. A., et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis., 2013; 13 (9): 785–796. https://doi:10.1016/S1473-3099(13)70190-7.
21. Patel, G., and Bonomo, R. A. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol., 2013; 4: 48. https://doi:10.3389/fmicb.2013.00048.
22. Doi, Y., and Paterson, D. L. Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med, 2015; 36: 74-84. https://doi:10.1055/s-0035-1544208.
23. Cheng, L., Cao, X. L., Zhang, Z. F., et al. Clonal dissemination of KPC-2 producing *Klebsiella pneumoniae* ST11 clone with high prevalence of oqxAB and rmtB in a tertiary hospital in China: results from a 3-year period. Ann Clin Microbiol Antimicrob. 2016; 15: 1. https://doi:10.1186/s12941-015-0109-x.
24. Guducuoglu, H., Gursoy, N. C., Yakupogullari, Y., et al. Hospital Outbreak of a Colistin-Resistant, NDM-1- and OXA-48-Producing *Klebsiella pneumoniae*: High Mortality from Pandrug Resistance. Microb Drug Resist. 2018; 24 (7): 966–972. https://doi:10.1089/mdr.2017.0173.
25. Tsai, Y. K., Fung, C. P., Lin, J. C., et al. *Klebsiella pneumoniae* outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2011; 55 (4): 1485–1493. https://doi:10.1128/AAC.01275-10.
26. Hamzaoui, Z., Ocampo-Sosa, A., Maamar, E., et al. An Outbreak of NDM-1-Producing *Klebsiella pneumoniae*, Associated with OmpK35 and OmpK36 Porin Loss in Tunisia. Microb Drug Resist. 2018; 24: 1137–1147. https://doi:10.1089/mdr.2017.0165.
27. Zhou, H., Zhang, K., Chen, W., et al. Epidemiological characteristics of carbapenem-resistant Enterobacteriaceae collected from 17 hospitals in Nanjing district of China. Antimicrob Resist Infect Contr. 2020; 9 (1): 15. https://doi:10.1186/s13756-019-0674-4.
28. Boucher, H. W., Talbot, G. H., Bradley, J. S., et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009; 48 (1): 1–12. https://doi.org/10.1086/595011.
29. Weiner-Lastinger, L. M., Abner, S., Edwards, J. R., et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. Infect Control Hosp Epidemiol. 2020; 41 (1): 1–18. https://doi.org/10.1017/ice.2019.296.
30. Abebe, M., Tadesse, S., Meseret, G., and Derbie, A. Type of bacterial isolates and antimicrobial resistance profile from clinical samples at a referral hospital, Northwest Ethiopia: five years data analysis. BMC Res Notes. 2019; 12 (1): 568. https://doi.org/10.1186/s13104-019-4604-6.
31. Ogbolu, D. O., and Webber, M. A. High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. Int J Antimicrob Agents. 2014; 43 (5): 412-417. https://doi.org/10.1016/j.ijantimicag.2014.01.014
32. Kehinde, A. O., Ademola, S. A., Okesola, A. O., Oluwatosin, O. M., and Bakare, R. A. Pattern of bacterial pathogens in burn wound infections in Ibadan, Nigeria. Ann Burns and Fire Disasters. 2004; 17 (1): 1-7. https://repository.ui.edu.ng/handle/123456789/10061
33. Olowe, O. A., Okanlawon, B. M., Olowe, R. A., and Olayemi, A. B. Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, southwestern Nigeria. Afr J Microbiol Res. 2008; 2: 8–11.
34. Nsofor, C. A., Iroegbu, C. U., Call, D. R., and Davies, M. A. Detection of antibiotic resistance genes of *Escherichia coli* from domestic livestock in southeast Nigeria with DNA microarray. J Cell Anim Biol. 2013; 7 (12): 149–163.
35. Trindade, L. C., Marques, E., Lopes, D. B., and Ferreira, M. A. S. V. Development of a molecular method for detection and identification of *Xanthomonas campestris* pv. *viticola*. Summa Phytopathologica. 2007; 33 (1): 16-23. https://doi.org/10.1590/S0100-54052007000100002
36. Cheesbrough, M. Microbiological Tests. In: District Laboratory Practice in Tropical Countries. Cambridge University Press. 2010: 64-65.
37. Ezeador, C. O., Ejikeugwu, P. C., Ushie, S. N., and Agbakoba, N. R. Isolation, Identification and Prevalence of *Pseudomonas aeruginosa* Isolates from Clinical and Environmental Sources in Onitsha Metropolis, Anambra State. Euro J Med Health Sci. 2020; 2 (2). https://doi.org/10.24018/ejmed.2020.2.2.188.
38. Holmes, B., Willcox, W. R., and Lapage, S. P. Identification of Enterobacteriaceae by the API 20E system. J Clin Pathol. 1978; 31: 22 - 30.
39. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. VET01S. 30th ed. Wayne (PA) 2020: Clinical and Laboratory Standards Institute.
40. Handal, R., Qunibi, L., Sahouri, I., et al. Characterization of Carbapenem-Resistant *Acinetobacter baumannii* Strains Isolated from Hospitalized Patients in Palestine. Int J Microbiol. 2017; 2017: 8012104. https://doi.org/10.1155/2017/8012104.
41. Mushi, M. F., Mshana, S. E., Imirzalioglu, C., and Bwanga, F. Carbapenemase genes among multidrug resistant gram-negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed Res Int. 2014; 2014: 303104. https://doi.org/10.1155/2014/303104
42. Aly, M., and Balkhy, H. H. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. Antimicrob Resist Infect Control. 2012; 1 (1): 26. https://doi.org/10.1186/2047-2994-1-26.
43. Eltahawy, A. T., and Khalaf, R. M. Antibiotic resistance among Gram-negative non-fermentative bacteria at a teaching hospital in Saudi Arabia. J Chemother. 2001; 13 (3): 260-264. https://doi.org/10.1179/joc.2001.13.3.260.

44. Balkhair, A., Al-Farsi, Y. M., Al-Muharrmi, Z., et al. Epidemiology of Multi-Drug- Resistant Organisms in a Teaching Hospital in Oman: A One-Year Hospital-Based Study. Sci World J. 2014; Article ID 157102, 6 pages http://dx.doi.org/10.1155/2014/157102
45. Jaggi, N., Sissodia, P., and Sharma, L. Control of multidrug resistant bacteria in a tertiary care hospital in India. Antimicrob Resist Infect Control. 2012; 1 (1): 23. https://doi.org/10.1186/2047-2994-1-23.
46. El-Saed, A., Balkhy, H. H., Al-Dorzi, H. M., Khan, R., Rishu, A. H., and Arabi, Y. M. *Acinetobacter* is the most common pathogen associated with late-onset and recurrent ventilator-associated pneumonia in an adult intensive care unit in Saudi Arabia. Int J Infect Dis. 2013; 17 (9): e696-701. https://doi.org/10.1016/j.ijid.2013.02.004.
47. Chang, Y. J., Yeh, M. L., Li, Y. C., et al. Predicting hospital-acquired infections by scoring system with simple parameters. PLoS One. 2011; 6 (8): e23137. https://doi.org/10.1371/journal.pone.0023137.
48. Gurung, S., Kafle, S., Dhungel, B., et al. Detection of OXA-48 Gene in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* from Urine Samples. Infect Drug Resist. 2020; 13: 2311-2321. https://doi.org/10.2147/IDR.S259967.
49. Chen, D., Li, H., Zhao, Y., et al. Characterization of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Fuzhou, China. J Appl Microbiol. 2020; 129 (5): 1220-1226. https://doi.org/10.1111/jam.14700
50. Mohapatra, S., Kumar, A., Bir, R., et al. Use of Ertapenem as a Marker for Detection of Carbapenem Resistance for Enterobacteriaceae. J Clin Diagn Res. 2020; 14 (12): DC05-DC08. https://www.doi.org/10.7860/JCDR/2020/46140/14271.
51. Bitew, A., and Tsige, E. High prevalence of multidrug-resistant and extended-spectrum β-lactamase-producing Enterobacteriaceae: a cross-sectional study at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia. J Trop Med. 2020; 2020: 6167234. https://doi:10.1155/2020/6167234.
52. Eshetie, S., Unakal C., Gelaw A., Ayelign, B., Endris, M., and Moges, F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. Antimicrob Resist Infect Contr. 2015; 4: 12. https://doi:10.1186/s13756-015-0054-7.
53. Nikaido, H. Multidrug resistance in bacteria. Annu Rev Biochem. 2009; 78 (1): 119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923
54. Odonkor, S. T., and Addo, K. K. Prevalence of Multidrug-Resistant *Escherichia coli* Isolated from Drinking Water Sources. Int J Microbiol. 2018; 2018: 7204013. https://doi.org/10.1155/2018/7204013.
55. Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., and Olayinka, B. O. High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. Germs. 2020; 10 (4): 310-321. https://doi.org/10.18683/germs.2020.1223.
56. Mohammed, Y., Zailani, S., and Onipede, A. Characterization of KPC, NDM and VIM Type Carbapenem Resistance Enterobacteriaceae from North Eastern, Nigeria. J Biosci Med. 2015; 3: 100-107. https://doi.org/10.4236/jbm.2015.311013.
57. Tsai, Y. M., Wang, S., Chiu, H. C., Kao, C. Y., and Wen, L. L. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiol. 2020; 20 (1): 315. https://doi.org/10.1186/s12866-020-02010-3.
58. Aminu, A., Daneji, I. M., Yusuf, M. A., et al. Carbapenem-resistant Enterobacteriaceae infection among patients admitted to intensive care units in Kano, Nigeria. Sahel Med J. 2021; 24: 1-9. https://doi.org/10.4103/smj.smj_14_20.
59. Abdurasheed, A. B., Alade, T. O., Olorode, O. A., and Joy, P. A. Prevalence of Metallo-β-lactamase (*bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{NDM}$) Genes among Clinical Isolates from Two Tertiary Hospitals in Yenagoa, Bayelsa State, Nigeria. Int J Res Innov Appl Sci. 2019; 4 (12): 153.

Fadare et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 320 - 329 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2485. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Molecular detection of tetracycline resistance genes in an emerging *Enterobacteriaceae* isolate from earthen catfish ponds in Akure, Nigeria

*Fadare, O. S., Olalekan, P., and *Salam, L. B.

Department of Biological Sciences (Microbiology Unit), Faculty of Basic and Applied Sciences, Elizade University, Wuraola Ade Ojo Avenue, P. M. B. 002, Ilara-Mokin, Ondo State, Nigeria
*Correspondence to: olalekan.fadare@elizadeuniversity.edu.ng; +2348169001041; ORCiD: 0000-0002-1749-2238
*Correspondence to: lateef.salam@elizadeuniversity.edu.ng; +234 810 245 0606; ORCID: 0000-0001-8353-534X

Abstract:

Background: Aquaculture environments are increasingly recognized as reservoirs for antimicrobial-resistant bacteria due to the routine use of antibiotics such as tetracyclines. Members of the family *Enterobacteriaceae* readily acquire and disseminate resistance determinants, posing risks to food safety and public health. In Nigeria, molecular data on tetracycline resistance in aquaculture systems remain limited. This study investigated the occurrence and molecular basis of tetracycline resistance among *Enterobacteriaceae* isolated from earthen catfish ponds in Akure, Nigeria.
Methodology: Composite samples were collected from five different locations within each of two randomly selected commercial-scale earthen catfish ponds and pooled for analysis. The resulting samples were cultured on selective media. Antibiotic susceptibility of four representative isolates was performed by the Kirby–Bauer disc diffusion method against ampicillin, cefuroxime, ciprofloxacin and tetracycline. The isolate exhibiting phenotypic tetracycline resistance was further characterized by 16S rRNA gene sequencing and phylogenetic analysis. Polymerase chain reaction (PCR) assay was used to detect tetracycline resistance genes (*tetA* and *tetM*) in the isolate.
Results: All four representative isolates were fully resistant to ampicillin and cefuroxime but susceptible to ciprofloxacin. One isolate (EP9) displayed complete resistance to tetracycline. Molecular identification revealed EP9 as *Escherichia albertii*, confirmed by phylogenetic clustering within a distinct *E. albertii* clade. PCR analysis detected the presence of the *tetA* gene (210 bp) in EP9, while *tetM* was not detected.
Conclusion: This study provides molecular evidence that earthen catfish ponds in Akure harbour tetracycline-resistant *Escherichia albertii*. The detection of the plasmid-mediated *tetA* gene underscores the role of aquaculture systems as reservoirs and dissemination hubs for clinically relevant antimicrobial resistance genes, highlighting the need for strengthened antibiotic stewardship and routine AMR surveillance in aquaculture.

Keywords: *Escherichia albertii,* Enterobacteriaceae, Aquaculture tetracycline resistance, Nigeria

Received Dec 25, 2025; Revised Feb 24, 2026; Accepted Feb 27, 2026

Détection moléculaire des gènes de résistance à la tétracycline chez un isolat émergent d'*Enterobacteriaceae* provenant d'étangs piscicoles de poissons-chats à fond de terre à Akure, Nigeria

*Fadare, O. S., Olalekan, P., et *Salam, L. B.

Département des Sciences Biologiques (Unité de Microbiologie), Faculté des Sciences, Fondamentales et Appliquées, Université Elizade, Wuraola Ade Ojo Avenue, P. M. B. 002, Ilara-Mokin, État d'Ondo, Nigéria
*Correspondance à: olalekan.fadare@elizadeuniversity.edu.ng; +2348169001041; ORCiD: 0000-0002-1749-2238
*Correspondance à: salam.lateef@elizadeuniversity.edu.ng; +2348102450606; ORCID: 0000-0001-8353-534X

Résumé:

Contexte: Les environnements aquacoles sont de plus en plus reconnus comme des réservoirs de bactéries résistantes aux antimicrobiens en raison de l'utilisation courante d'antibiotiques tels que les tétracyclines. Les membres de la famille des *Enterobacteriaceae* acquièrent et disséminent facilement des déterminants de résistance, ce qui pose des risques pour la sécurité alimentaire et la santé publique. Au Nigeria, les données moléculaires sur la résistance aux tétracyclines dans les systèmes aquacoles restent limitées. Cette étude a examiné la présence et la base moléculaire de la résistance à la tétracycline chez des *Enterobacteriaceae* isolées d'étangs piscicoles de poissons-chats à fond de terre à Akure, au Nigeria.
Méthodologie: Des échantillons composites ont été prélevés à partir de cinq emplacements différents dans chacun de deux étangs piscicoles commerciaux à fond de terre sélectionnés au hasard et regroupés pour analyse. Les échantillons obtenus ont été cultivés sur des milieux sélectifs. La sensibilité aux antibiotiques de quatre isolats représentatifs a été déterminée par la méthode de diffusion sur disque de Kirby–Bauer contre l'ampicilline, le céfuroxime, la ciprofloxacine et la tétracycline. L'isolat présentant une résistance phénotypique à la tétracycline a ensuite été caractérisé par le séquençage du gène de l'ARNr 16S et une analyse phylogénétique. Une réaction en chaîne par polymérase (PCR) a été utilisée pour détecter les gènes de résistance à la tétracycline (*tetA* et *tetM*).
Résultats : Les quatre isolats représentatifs étaient entièrement résistants à l'ampicilline et au céfuroxime, mais sensibles à la ciprofloxacine. Un isolat (EP9) a montré une résistance complète à la tétracycline. L'identification moléculaire a révélé que l'isolat EP9 correspondait à *Escherichia albertii*, confirmé par son regroupement phylogénétique au sein d'un clade distinct de *E. albertii*. L'analyse PCR a détecté la présence du gène *tetA* (210 pb) chez EP9, tandis que le gène *tetM* n'a pas été détecté.
Conclusion: Cette étude fournit des preuves moléculaires indiquant que les étangs piscicoles à fond de terre d'Akure abritent *Escherichia albertii* résistante à la tétracycline. La détection du gène *tetA* médié par plasmide souligne le rôle des systèmes aquacoles comme réservoirs et centres de dissémination de gènes de résistance aux antimicrobiens cliniquement pertinents, mettant en évidence la nécessité de renforcer la gestion des antibiotiques et la surveillance régulière de la RAM dans l'aquaculture.

Mots-clés: *Escherichia albertii*, *Enterobacteriaceae*, aquaculture, résistance à la tétracycline, Nigeria.

Introduction:

Antimicrobial resistance (AMR) is one of the most urgent global public health threats of the 21st century, undermining the effectiveness of antibiotics and compromising the treatment of infectious diseases in both humans and animals. In 2019 alone, bacterial AMR was estimated to have caused approximately 1.27 million deaths worldwide (1), with projections suggesting that annual mortality could rise to 10 million by 2050 if current trends persist. Among bacterial groups of concern, members of the family *Enterobacteriaceae* are notable for their capacity to acquire and disseminate resistance determinants, often through mobile genetic elements such as plasmids and integrons.

The global escalation of multidrug-resistant *Enterobacteriaceae* has been reported in diverse environments, including clinical settings (2), agricultural areas (3), and aquatic ecosystems (4), where antibiotic use and environmental exposure contribute to resistance selection and propagation. Aquaculture, particularly in low- and middle-income countries, often employs antibiotics such as oxytetracycline for disease control and growth promotion. This practice exerts strong selective pressure on indigenous bacterial communities, thereby enhancing the persistence of antibiotic resistance genes (ARGs) in pond water, sediments, and cultured fish.

Tetracyclines are widely used in aquaculture due to their broad-spectrum activity and low cost; however, extensive application has been strongly linked to the emergence of tetracycline resistance and the widespread occurrence of *tet* genes in aquaculture environments, as evidenced by molecular studies using PCR-based detection methods (5).

Studies in fish farms and aquaculture environments have reported the occurrence of multiple *tet* genes among diverse bacterial taxa, including *Aeromonas*, *Pseudomonas* and *Enterobacteriaceae*, highlighting aquaculture systems as reservoirs and hotspots for tetracycline resistance determinants (6,7). Furthermore, the persistence of tetracycline resistance genes in pond environments appears to be influenced not only by ongoing antibiotic use but also by the intrinsic stability of resistance gene pools and horizontal gene transfer mechanisms (8). In Nigeria, investigations into antibiotic resistance among bacteria isolated from aquaculture environments remain limited, despite documented resistance to multiple antibiotics in related settings such as water distribution systems and animal production environments (9).

Given the economic and nutritional importance of catfish farming in southwestern Nigeria, understanding the molecular epidemiology of tetracycline resistance in *Enterobacteriaceae* from earthen ponds is essential for informing antibiotic stewardship, food safety, and public health risk assessments. This study, therefore, aimed to detect and characterize tetracycline resistance genes among *Enterobacteriaceae*

isolates from earthen catfish ponds in Akure, Nigeria, using PCR methodologies.

Materials and method:

Study area and sample collection:

Two commercial-scale earthen ponds used for catfish culture in Akure, Ondo State, Nigeria, were randomly selected for this study in May 2025. Water samples were collected from five different points within each pond to obtain representative coverage of the pond environment and minimize sampling bias caused by spatial variation in microbial distribution. Equal volumes of water from the five locations in each pond were pooled to form a composite sample and collected aseptically into sterile sampling bottles. The composite sampling approach was adopted to provide an overall representation of the microbial quality of each pond rather than conditions at a single point.

Immediately after collection, the samples were placed in an insulated container containing ice packs to maintain low temperature and limit changes in microbial composition during transport. The samples were transported promptly to the laboratory and processed within two hours of collection to ensure the reliability and integrity of the microbiological analysis.

Culture isolation and biochemical identification of *Enterobacteriaceae*:

Water samples were serially diluted up to 10^{-8} using sterile physiological saline, and 100 µl of each dilution was inoculated onto Chromogenic Coliform Agar (Oxoid, UK). Plates were incubated at 35°C and examined after 24 and 48 hours. The medium enables the presumptive differentiation of members of the *Enterobacteriaceae* based on chromogenic substrates that detect β-galactosidase and β-glucuronidase enzyme activities. After incubation, colonies were examined for characteristic colour reactions and morphological features. According to the manufacturer's colour chart for Chromogenic Coliform Agar, *Escherichia coli* colonies appeared dark blue to violet, while other coliform bacteria produced pink to red or Salmon red colonies. Non-coliform members of the *Enterobacteriaceae* appeared as colourless or pale colonies. Colony characteristics including size, shape, elevation, margin and surface appearance were also noted. From each plate, four colonies with distinct colour and morphological characteristics were randomly selected and purified by repeated streaking on nutrient agar to obtain pure cultures.

The purified isolates were Gram stained to confirm that the isolates were Gram-negative rods, consistent with members of the *Enterobacteriaceae* family. Further phenotypic identification was carried out using standard biochemical tests, including oxidase, catalase, indole production, methyl red (MR), Voges–Proskauer (VP), citrate utilization, urease, and motility tests as well as Triple Sugar Iron (TSI) agar test for carbohydrate fermentation and hydrogen sulphide production.

Antimicrobial susceptibility testing of isolates:

The antibiotic susceptibility of the purified isolates was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK) in triplicate, following CLSI guidelines. The antibiotics tested included tetracycline (30µg), cefuroxime (30µg), ciprofloxacin (5µg), and ampicillin (10µg). After incubation at 37°C for 24 hours, inhibition zone diameters were measured, and each isolate was categorized as susceptible, intermediate, or resistant based on CLSI interpretative criteria supplied with the antibiotic discs (Himedia). The isolate that was fully resistant to tetracycline was selected for further study.

16S rRNA gene sequencing and phylogenetic analysis:

Molecular identification of the tetracycline-resistant isolate was carried out through 16S rRNA gene sequencing. Genomic DNA was extracted and used as the template for PCR amplification. The PCR reaction was prepared using the Solis Biodyne 5X HOT FIREPol® Blend Master Mix. Each 25 µl reaction contained 1X Blend Master Mix buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP (Solis Biodyne), 25 pmol of each primer (BIOMERS, Germany), 2 units of HOT FIREPol® DNA polymerase with proofreading activity, 5 µl of template DNA, and sterile distilled water to volume. Amplification was performed in a Techne (3Prime series) thermal cycler with an initial denaturation at 95°C for 15 min, followed by 35 cycles of 95°C for 30 s, 61°C for 1 min, and 72°C for 1 min 30 s, and a final extension at 72°C for 10 min. The resulting amplicons were sequenced using the Nimagen BrilliantDye™ Terminator v3.1 Cycle Sequencing Kit (BRD3-100/1000) following the manufacturer's instructions. Sequence chromatograms were examined and edited using FinchTV software.

Taxonomic identification of the isolate was carried out by comparative analysis of its 16S rRNA gene sequence using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic analysis was performed using homologous sequences

retrieved from the NCBI Gene/Nucleotide database and inferred in MEGA version 12.1 using the Maximum Likelihood method. The tree was constructed using 1,000 bootstrap replicates, with bootstrap values indicating the robustness of the inferred relationships.

Detection of *tetA* and *tetM:*

PCR assays targeted tetracycline resistance genes using specific primers (Table 1). Conditions included an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. Products were analyzed on 1.5% agarose gels stained with ethidium bromide.

Results:

Frequency of *Enterobacteriaceae* isolated from earthen pond:

A total of two commercial-scale earthen catfish ponds were sampled in this study. From each pond, water samples were collected from five different locations and pooled to obtain one composite sample per pond. Microbiological analysis of the composite samples yielded a total of 103 colonies on Chromogenic Coliform Agar at a dilution factor of 10^{-7} (Table 2). From Pond 1, a total of 55 colonies were recovered. Among these, 15 colonies appeared dark blue to violet, presumptively indicating *Escherichia coli*, while 17 colonies appeared pink to red, representing other coliform bacteria. In addition, 11 colonies displayed salmon to red pigmentation, whereas 12 colonies appeared colourless or pale, suggesting non-coliform members of the *Enterobacteriaceae*.

Similarly, 48 colonies were recovered from Pond 2. Of these, 11 colonies were dark blue to violet (presumptive *E. coli*), 12 colonies were pink to red (other coliform bacteria), 15 colonies appeared salmon to red, and 10 colonies were colourless or pale, consistent with non-coliform *Enterobacteriaceae*. Overall, the chromogenic reactions observed across both ponds indicate the presence and distribution of different *Enterobacteriaceae* groups in the earthen pond water, with coliform bacteria representing the predominant colony types.

Table 1: PCR primers used in this study

Target	Primer sequence (5'–3')	Annealing temp (°C)	Product size (bp)
tetA	F: GCTACATCCTGCTTGCCTTC	55	210
	R: CATAGATCGCCGTGAAGAGG		
tetM	F: CGAGGTCCGTCTGAACTTTGC	55	583
	R: GCGGCACTTCGATGTGAATGGT		

Table 2: Chromogenic colony distribution of *Enterobacteriaceae* isolates from earthen catfish pond water

Pond ID	Number of sampling points	Composite samples collected	*Number of colonies	Colony type
Pond 1	5	1	55	15 dark blue/violet, 17 pink/red, 11 Salmon to red, 12 colourless/pale ((non-coliform)
Pond 2	5	1	48	11 dark blue/violet, 12 pink/red, 15 Salmon to red, 10 colourless/pale ((non-coliform)

*Colonies recovered from dilution factor of 10^{-7}

Table 3: Mean zone of inhibition and antibiotic sensitivity pattern of the selected bacterial isolates

Antibiotics	Mean Zones of inhibition (mm) ± SD			
	EP1	EP2	EP3	EP9
Ciprofloxacin (5 µg)	30.4 ± 1.3 (S)	24.5 ± 1.9 (S)	25.2 ± 1.1 (S)	32.4 ± 1.6 (S)
Ampicillin (10 µg)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)
Tetracycline (30 µg)	15.4 ± 1.4 (I)	16.3 ± 1.2 (I)	12.4 ± 1.2 (I)	0.0 ± 0.0 (R)
Cefuroxime (30 µg)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)	0.0 ± 0.0 (R)

Values are presented as mean ± standard deviation of triplicate determinations (n = 3). Antibiotic susceptibility interpretation was performed according to the Clinical and Laboratory Standards Institute (CLSI). Key: S = Sensitive; I = Intermediate; R = Resistant.

Antibiotic susceptibility of isolates:

The antibiotic susceptibility of the four selected bacterial isolates (EP1, EP2, EP3, and EP9), as shown in Table 3, indicated significant differences in their responses to the tested antibiotics. All the four isolates were sensitive to ciprofloxacin with isolate EP9 having highest mean zone diameter of inhibition and isolate EP2 with the lowest.

All four isolates, however, were resistant to ampicillin and cefuroxime, as shown by the absence of inhibitory zones. Isolate EP9 was resistant to tetracycline with no zone of inhibition, while isolates EP1, EP2, and EP3 showed intermediate sensitivity to tetracycline.

Culture isolation and biochemical identification of representative *Enterobacteriaceae*:

The biochemical and sugar fermentation profiles of the four representative isolates are presented in Table 4. The motility test for the isolate designated EP9 did not show visible growth radiating from the point of inoculation, unlike the other isolates, and was therefore considered negative.

Table 4: Sugar fermentation and biochemical characteristics of selected bacterial isolates from earthen catfish ponds

Test	EP1	EP2	EP3	EP9
Gram reaction	– (rod)	– (rod)	– (rod)	– (rod)
Oxidase	+	–	–	–
Catalase	+	+	+	+
Motility	+	+	+	–
Indole	–	+	–	+
Citrate utilization	–	–	+	–
Urease	–	–	–	–
Methyl red	+	+	+	+
Voges–Proskauer	–	–	–	–
TSI (slant/butt)	A/A	A/A	K/A + H_2S	A/A
Gas production	+	+	–	–
H_2S production	–	–	+	–
Sugar fermentation				
Glucose	+	+	+	+
Lactose	–	+	–	–
Sucrose	+	+	–	–
Mannitol fermentation	+	+	+	+
Presumptive identity	*Aeromonas* spp.	*Escherichia coli*	*Salmonella* spp.	*Escherichia* spp

Key: A/A = Acid slant / Acid butt (sugar fermentation); **K/A** = Alkaline slant / Acid butt; **+** = Positive reaction; **–** = Negative reaction **H_2S** = Hydrogen sulphide production

Molecular identification and phylogenetic relationship of the tetracycline-resistant isolate: The isolate designated EP9 was identified based on the highest sequence similarity and alignment score with reference sequences in the database. BLAST analysis revealed that isolate EP9 showed the closest phylogenetic affiliation with *Escherichia albertii*. The 16S rRNA gene sequence of isolate EP9 has been deposited in the NCBI nucleotide sequence database (GenBank) under the accession number PX312839.1.

The resulting phylogenetic tree (Fig. 2) resolved the analyzed sequences into two well-defined major clades corresponding to *Escherichia albertii* and *Escherichia coli* lineages. The Maximum Likelihood phylogenetic tree showed that isolate EP9 PX312839.1 clustered within the *Escherichia albertii* clade, grouping closely with reference *E. albertii* strains and clearly separated from *Escherichia coli* sequences. This confirms the taxonomic placement of the isolate as *Escherichia albertii*.

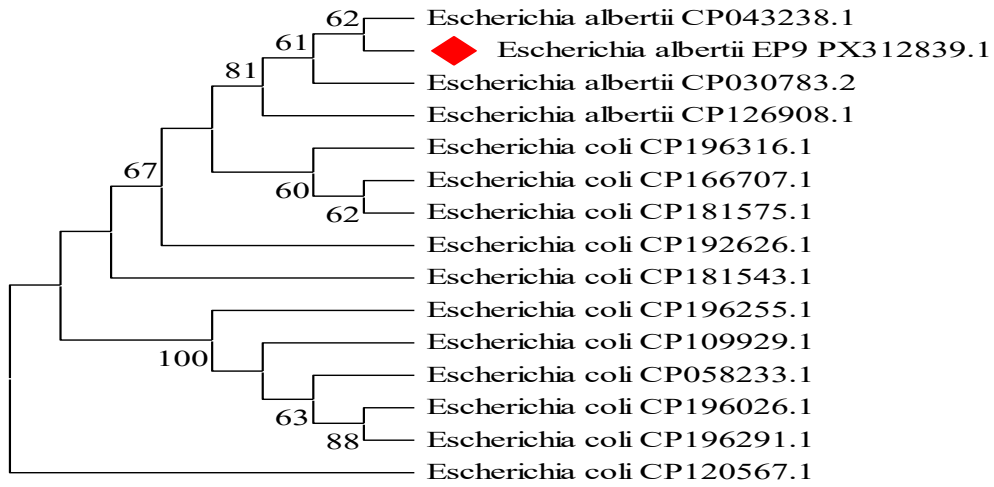
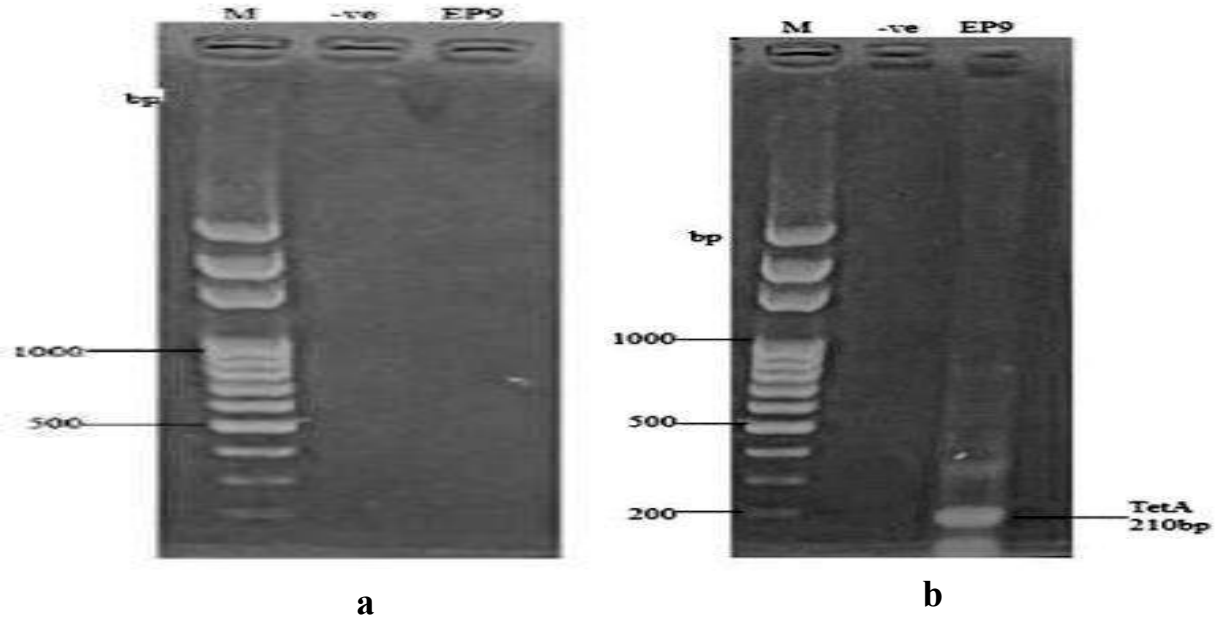


Fig 1: Phylogenetic tree illustrating the evolutionary relationship between the *Escherichia albertii* EP9 sequence identified in this study and closely related *Escherichia* spp. sequences retrieved from the NCBI GenBank database.



a b

Fig 2: Agarose gel electrophoresis of PCR products for tetracycline resistance genes. M: DNA marker; –ve: negative control; EP9: test isolate. **(a)** shows absence of the *tetM* gene (583 bp), **(b)** shows presence of the *tetA* gene (210 bp)

PCR detection of tetracycline resistance genes:

Agarose gel electrophoresis of the PCR amplified products showed no detectable amplicon corresponding to the *tetM* gene, indicating a negative result for this determinant. In contrast, PCR amplification targeting the *tetA* gene produced a clear and distinct band at approximately 210 bp, consistent with the expected amplicon size for *tetA* (Fig. 2). These results confirm the presence of the *tetA* gene and the absence of *tetM* in the tested isolate.

Discussion:

The emergence and dissemination of antimicrobial resistance (AMR) in environmental and clinical bacteria constitute a major global public health concern. In this study, a tetracycline-resistant isolate (EP9) was recovered from an earthen fish pond in Akure, Ondo State, Nigeria, an aquaculture setting recognized as a potential hotspot for resistant bacteria due to routine antibiotic application. Molecular analysis identified the isolate as *Escherichia albertii*, an emerging zoonotic pathogen.

The antibiotic susceptibility profile of isolate EP9 reveals a classical multidrug-resistant (MDR) phenotype, defined by resistance to three or more antimicrobial classes (10), which in this case included ampicillin, cefuroxime and tetracycline. Complete resistance to ampicillin and cefuroxime, alongside acquired tetracycline resistance, mirrors patterns commonly reported among members of the family *Enterobacteriaceae* from aquatic and environmental sources (11,12). The universal resistance to ampicillin (a penicillin) and cefuroxime (a second-generation cephalosporin) strongly suggests the production of broad-spectrum β-lactamases, most plausibly TEM-1 or SHV-1 enzymes, which remain among the most prevalent β-lactamases in *Enterobacteriaceae* worldwide (13,14).

In contrast, the retained susceptibility of EP9 to ciprofloxacin, a fluoroquinolone, is notable but potentially transient. Fluoroquinolone resistance is increasingly mediated by plasmid-encoded determinants such as *qnr* genes, *aac(6′)-Ib-cr*, and efflux systems, which often co-localize with β-lactam and tetracycline resistance genes on mobile genetic elements (15, 16). The coexistence of ciprofloxacin susceptibility with an otherwise MDR phenotype may therefore represent an intermediate evolutionary stage preceding the acquisition of full fluoroquinolone resistance. This observation reinforces the need for sustained AMR surveillance and prudent fluoroquinolone use in both clinical and agricultural contexts to preserve the efficacy of this critical antimicrobial class (17,18).

A key strength of this study lies in the precise taxonomic resolution of the tetracycline-resistant isolate. Using 16S rRNA gene sequencing and robust phylogenetic analysis, EP9 (accession number PX312839.1) clustered within a distinct monophyletic *E. albertii* clade, clearly separated from *E. coli*. This distinction elevates the significance of the findings beyond the commonly studied *E. coli* framework. Although *E. albertii* is increasingly recognized as an emerging enteropathogen responsible for human gastrointestinal disease (19,20), its role in environmental reservoirs of antimicrobial resistance remains poorly defined. Traditionally, *E. albertii* has been associated with clinical and foodborne contexts, but recent genomic investigations have begun to uncover its presence beyond clinical settings. A recent study reported the first genomic characterization of multidrug-resistant *E. albertii* isolated from retail fish, highlighting its occurrence in aquaculture-associated food sources and raising concerns about fish as a vector for the dissemination of pathogenic and resistant strains (21). The isolate exhibited phenotypic resistance to multiple antibiotics and harboured mobile genetic elements such as plasmids and transposons that facilitate gene exchange, suggesting potential for ARG transfer in food-related environments.

In addition to aquatic food sources, other studies have documented widespread multidrug resistance and ARG carriage in *E. albertii* from non-aquatic food products; for instance, poultry meat isolates exhibited high levels of resistance to clinically relevant antibiotics and harboured corresponding resistance genes identified by whole-genome sequencing (22). Older work on *E. albertii* from clinical and environmental sources similarly observed resistance to tetracycline and other antibiotics, indicating that this species is capable of acquiring and maintaining diverse resistance determinants (23). Collectively, these findings support the notion that *E. albertii*, like other members of *Enterobacteriaceae*, is not only a potential zoonotic pathogen but also a carrier of ARGs, including determinants for tetracyclines, β-lactams and other antimicrobial classes.

Molecular analysis identified the *tetA* gene as the detected determinant associated with tetracycline resistance in *E. albertii* EP9, while *tetM* was not observed. This resistance profile is consistent with the farmer's reported reliance on oxytetracycline as the primary anti-

microbial used in the pond, suggesting antibiotic-driven selection pressure in the aquaculture environment. Although the present analysis was limited to a subset of tetracycline resistance genes and does not exclude the possible presence of additional *tet* determinants, the targeted detection of *tetA*, one of the most prevalent and epidemiologically relevant tetracycline resistance genes in aquatic systems, represents a key strength of the study. Previous studies have shown that aquaculture systems with a history of oxytetracycline application exhibit significantly higher detection frequencies of tetracycline resistance genes, particularly *tetA,* than facilities without recent tetracycline exposure (5), underscoring the strong selective pressure exerted by antibiotic use in such environments. The *tetA* gene is a plasmid-encoded efflux pump that confers significant resistance to tetracycline. This gene, along with *tetB* are the most prevalent among tetracycline-resistant *Enterobacteriaceae* (24), allowing promiscuous dissemination between cells, often alongside other resistance genes, and contributing significantly to antibiotic resistance.

Within the context of this study conducted in Akure, the detection of *tetA* in the absence of *tetM* likely reflects both sustained oxytetracycline-driven selection and the ecological characteristics of earthen pond systems. These environments promote close interactions between bacterial communities in water and sediments, thereby facilitating horizontal gene transfer of mobile resistance determinants, especially plasmid-borne genes such as *tetA*. Notably, previous reports have emphasized that aquaculture settings can support the persistence and dissemination of antibiotic resistance genes even in the absence of detectable antibiotic residues, owing to the stability of environmental resistance gene pools and continuous microbial exchange (10).

Globally, investigations of freshwater aquaculture systems consistently identify tetracycline resistance genes among the most prevalent AMR determinants. A meta-analysis has revealed strong association between environmental tetracycline concentrations and the abundance of *tet* genes, including *tetA*, reinforcing the role of ponds as long-term reservoirs of resistance elements (25). Similarly, studies from commercial fin-fish culture systems in India reported *tetA* as the dominant resistance gene among heterotrophic bacteria isolated from water, sediment, and fish tissues, further supporting the widespread distribution of this efflux-mediated resistance mechanism in aquaculture settings subjected to tetracycline exposure (26). Earthen aquaculture ponds provide conditions conducive to the persistence and horizontal dissemination of resistance genes, owing to high organic load, dense microbial populations, and sustained environmental exposure.

The *tetA* gene encodes an energy-dependent efflux pump that actively exports tetracycline from the bacterial cell, thereby reducing intracellular drug accumulation. This gene is typically plasmid-borne, facilitating horizontal gene transfer among diverse bacterial taxa. In contrast, *tetM* encodes a ribosomal protection protein commonly associated with conjugative transposons. The absence of *tetM* in EP9 suggests selective enrichment of plasmid-mediated efflux mechanisms within this ecological niche, potentially driven by co-selection with other plasmid-encoded resistance determinants. Collectively, these findings indicate that aquaculture environments not only select for tetracycline resistance but also favour genetic platforms that enhance the dissemination of multidrug resistance within environmental bacterial communities.

Conclusion:

This study demonstrates that earthen catfish ponds in Akure, Nigeria, serve as environmental reservoirs of multidrug-resistant *Enterobacteriaceae* and harbour clinically relevant tetracycline resistance determinants. The recovery and molecular characterization of a tetracycline-resistant *E. albertii* isolate carrying the plasmid-mediated *tetA* gene highlight the expanding ecological niche of this emerging zoonotic pathogen beyond clinical and foodborne settings into aquaculture environments. The absence of *tetM* alongside the presence of *tetA* suggests selective enrichment of efflux-mediated resistance mechanisms, likely driven by sustained oxytetracycline use and facilitated by horizontal gene transfer within pond microbial communities.

The detection of multidrug resistance in an aquaculture-associated *E. albertii* strain raises important public health concerns, given the potential for transmission through fish consumption, occupational exposure, and environmental dissemination. These findings reinforce the role of aquaculture systems as interfaces linking environmental, animal, and human reservoirs of antimicrobial resistance. Collectively, this work provides molecular evidence that underscores the need for routine surveillance of resistance genes in aquaculture settings, stricter regulation of antibiotic use, and the adoption of alternative disease management strategies such as vaccination, probiotics, and improved farm hyg-

iene. Strengthening antimicrobial stewardship in aquaculture is essential to limit the persistence and spread of resistance determinants and to safeguard both food safety and public health.

Contribution of authors:

FOS, OP and SLB conceptualized and designed the study. FOS and OP were involved in data collection. FOS, OP and SLB were involved in the writing and revising the manuscript for intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Source of funding:

No funding was obtained for this study

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Murray, C. J. L., Ikuta, K. S., Sharara, F., et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022; 399 (10325):629–655. https://doi.org/10.1016/s0140-6736(21)02724-0
2. Seguni, N. Z., Kimera, Z. I., Msafiri, F., et al. Multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital sewage flowing through community sewage system and discharging into the Indian Ocean. Bulletin of the National Research Center. 2023; 47 (1). https://doi.org/10.1186/s42269-023-01039-4
3. Kamel, K., Merghad, A., Barour, D., Gherissi, D. E., and Khenenou, T. High antimicrobial resistance rates and multidrug resistance in *Enterobacteriaceae* isolates from poultry in Souk Ahras region, Algeria. Veterinary World. 2024; 17 (12): 2709–2718.: https://doi.org/10.14202/vetworld.2024.2709-2718
4. De Los Ríos, J. D., Párraga-Niño, N., Navarro, M., et al. Environmental Dispersion of Multiresistant *Enterobacteriaceae* in Aquatic Ecosystems in an Area of Spain with a High Density of Pig Farming. PubMed. 2025; 14 (8): https://pubmed.ncbi.nlm.nih.gov/40867949
5. Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A., and McMahon, K. D. Occurrence of Tetracycline Resistance Genes in Aquaculture Facilities with Varying Use of Oxytetracycline. Microbial Ecol. 2010; 59 (4): 799 – 807. https://doi.org/10.1007/s00248-009-9624-7
6. Hedayatianfard, K., Akhlaghi, M., and Sharifiyazdi, H. Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. PubMed. 2014; 5(4): 269 – 275. https://pubmed.ncbi.nlm.nih.gov/25610578
7. Cheng, X., Lu, Y., Song, Y., et al. Analysis of antibiotic resistance genes, environmental factors, and microbial community from aquaculture farms in five provinces, China. Front Microbiol. 2021; 12: 679805. https://doi.org/10.3389/fmicb.2021.679805
8. Tamminen, M., Karkman, A., Lõhmus, A., et al. Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environ Sci Technol. 2010; 45 (2): 386–391. https://doi.org/10.1021/es102725n
9. Adesoji, A. T., Ogunjobi, A. A., Olatoye, I. O., and Douglas, D. R. Prevalence of tetracycline resistance genes among multi-drug-resistant bacteria from selected water distribution systems in south-western Nigeria. Ann Clin Microbiol Antimicrob. 2015; 14 (1): 41. https://doi.org/10.1186/s12941-015-0093-1
10. Magiorakos, A. P., Srinivasan, A., Carey, R. B., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2011; 18 (3): 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
11. Akinbowale, O. L., Peng, H., Grant, P., and Barton, M. D. Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. Int J Antimicrob Agents. 2007; 30 (2): 177–182. https://doi.org/10.1016/j.ijantimicag.2007.03.012
12. Martinez, J. L., Fajardo, A., Garmendia, L., et al. A global view of antibiotic resistance. FEMS Microbiol Rev. 2008; 33 (1): 44–65. https://doi.org/10.1111/j.1574-6976.2008.00142.x
13. Bradford, P. A. Extended-Spectrum B-Lactamases in the 21st Century: Characterization, Epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001; 14 (4): 933–951. https://doi.org/10.1128/cmr.14.4.933-951.2001
14. Bush, K., and Jacoby, G. A. Updated functional classification of B-Lactamases. Antimicrob Agents Chemother. 2009; 54 (3): 969–976. https://doi.org/10.1128/aac.01009-09
15. Strahilevitz, J., Jacoby, G. A., Hooper, D. C., and Robicsek, A. Plasmid-Mediated Quinolone Resistance: a Multifaceted Threat. Clin Microbiol Rev. 2009; 22 (4): 664–689. https://doi.org/10.1128/cmr.00016-09
16. Jacoby, G. A., Strahilevitz, J., and Hooper, D. C. Plasmid-Mediated quinolone resistance. Microbiol Spectr. 2014; 2 (5). https://doi.org/10.1128/microbiolspec.plas-0006-2013
17. World Health Organization. Antimicrobial resistance: Global Report on Surveillance. 2014.
18. Food and Agriculture Organization of the United Nations (FAO). Monitoring and surveillance of antimicrobial resistance: Regional antimicrobial resistance monitoring and surveillance guidelines. Rome: FAO; 2019. https://www.fao.org/3/ca6897en/CA6897EN.pdf
19. Huys, G., Cnockaert, M., Janda, J. M, and Swings, J. *Escherichia albertii* sp. nov., a diarrhoeagenic species isolated from stool specimens of Bangladeshi children. Int J Syst Evol Microbiol. 2003; 53 (3): 807–810. https://doi.org/10.1099/ijs.0.02475-0
20. Muchaamba, F., Barmettler, K., Treier, A., Houf, K., and Stephan, R. Microbiology and epidemiology of *Escherichia albertii*—an emerging elusive foodborne pathogen. Microorganisms. 2022; 10(5): 875. http://doi.org10.3390/microorganisms10050875.
21. Srinivas, K., Ghatak, S., Milton, A. A. P., et al. Genomic characterization of multidrug-resistant *Escherichia albertii* of fish origin first isolation and insights into a potential food safety threat. Front Microbiol. 2025; 16: 1521202. https://doi.org/10.3389/fmicb.2025.1521202
22. Hassan, J., Utsho, K. S., Karmakar, S., et al. Occurrence and cross contamination of *Escherichia albertii* in retail chicken outlets in Bangladesh. Int J Food Microbiol. 2025; 431: 111081. https://doi.org/10.1016/j.ijfoodmicro.2025.111081

23. Li, Q., Wang, H., Xu, Y., et al. Multidrug-Resistant *Escherichia albertii*: Co-occurrence of β-Lactamase and MCR-1 Encoding Genes. Front Microbiol. 2018; 9: 258. https://doi.org/10.3389/fmicb.2018.00258
24. Jomehzadeh, N., Ahmadi, K., Ataee, N., and Afzali, M. Molecular detection of genes encoding resistance to tetracycline and quinolones among *Shigella* strains isolated from children with acute diarrhoea in southwest Iran. Iranian J Microbiol. 2023; 15 (5): 625–630. https://doi.org/10.18502/ijm.v15i5.13868.
25. Wang, C., Liu, X., Yang, Y., and Wang, Z. Antibiotic and antibiotic resistance genes in freshwater aquaculture ponds in China: A meta-analysis and assessment. J Cleaner Prod. 2021; 329: 129719. https://doi.org/10.1016/j.jclepro.2021.129719
26. Kusunur, A. B., Mogilipuri, S. S., Moturu, D., et al. Tetracycline resistance potential of heterotrophic bacteria isolated from freshwater fin-fish aquaculture system. J Appl Microbiol. 2023; 134 (4): https://doi.org/10.1093/jambio/lxad06

Thomas et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 330 - 338 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2505. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Assessment of genetic diversity in *Aspergillus carbonarius* through random amplified polymorphic DNA (RAPD) marker analysis

[1]Thomas, B. T., *[1]Coker, M. O., [1]Popoola, O. D., [1]Adesetan, T. O., [1]Banjo, O. A., [1]Taiwo, O. S., [1]Popoola, K. E., [2]Thomas, A. N., [1]Adegbe, G. M., and [3]Owolabi, S. L.

[1]Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
[2]Department of Animal Production, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
[3]Department of Science Laboratory Technology (Microbiology Unit), Gateway Polytechnic, Saapade, Ogun State, Nigeria
*Correspondence to: olawunmi.coker@oouagoiwoye.edu.ng; +2349054006625;
ORCID ID: https://orcid.org/0000-0003-0937-7755

Abstract:

Background: The importance of RAPD in revealing genetic diversity among ochratoxigenic strains of *Aspergillus carbonarius* lies in its ability to provide critical information that can support the development of effective strategies to limit ochratoxin A contamination in agricultural produce. This study therefore focused on evaluating the genetic diversity of 20 *A. carbonarius* strains previously isolated in an earlier investigation.
Methodology: This study focused on evaluating the genetic diversity of 20 *A. carbonarius* strains previously isolated in an earlier investigation. A total of 26 RAPD primers were initially screened to determine optimal annealing temperatures and to identify those that produced clear, consistent, and reproducible banding patterns. Primers meeting these criteria were subsequently employed in genetic analysis, following standard RAPD protocols.
Results: The obtained results revealed that 7 RAPD primers gave clear and good polymorphisms at the optimal PCR amplification conditions between 36°C and 40°C. These 7 primers generated 18 distinctive RAPD loci from the 20 *A. carbonarius* isolates. The analysis of population structure at the geopolitical region of Ogun State revealed that the intra-site genetic variation (range=0.02 to 0.33) made up most of the genetic variation observed (82.0%). The magnitude of the overall variation arising from site-level divergence (G_{ST}) ranged from 0.01 to 0.42, suggesting minimal site-level population structure. The phylogenetic relationships among the 20 *A. carbonarius* isolates revealed that none of the isolates exhibited genetically distinct clusters.
Conclusion: The high genetic variation detected among ochratoxigenic *A. carbonarius* strains underscores the biotechnological importance of RAPD-based molecular characterization for strain-level discrimination, biosafety assessment, and targeted ochratoxin A risk management, thereby supporting informed strain selection and application in fungal biotechnology.

Keywords: *Aspergillus carbonarius*; Ochratoxin; Genetic Diversity; RAPD

Received Mar 3, 2026; Revised Apr 11, 2026; Accepted Apr 12, 2026

Évaluation de la diversité génétique chez *Aspergillus carbonarius* grâce à l'analyse de marqueurs d'ADN polymorphe amplifié aléatoirement (RAPD)

[1]Thomas, B. T., *[1]Coker, M. O., [1]Popoola, O. D., [1]Adesetan, T. O., [1]Banjo, O. A., [1]Taiwo, O. S., [1]Popoola, K. E., [2]Thomas, A. N., [1]Adegbe, G. M., et [3]Owolabi, S. L.

[1]Département de Microbiologie, Université Olabisi Onabanjo, Ago-Iwoye, État d'Ogun, Nigeria
[2]Département de Production Animale, Université Olabisi Onabanjo, Ago-Iwoye, État d'Ogun, Nigeria
[3]Département de Technologie de Laboratoire Scientifique (Unité de Microbiologie), Polytechnique Porte, Saapade, État d'Ogun, Nigeria
*Correspondance à: olawunmi.coker@oouagoiwoye.edu.ng; +2349054006625;
ORCID: https://orcid.org/0000-0003-0937-7755

Résumé:

Contexte: L'importance de la technique RAPD pour révéler la diversité génétique des souches ochratoxigènes d'*Aspergillus carbonarius* réside dans sa capacité à fournir des informations cruciales pour l'élaboration de stratégies efficaces de limitation de la contamination des produits agricoles par l'ochratoxine A. Cette étude s'est donc concentrée sur l'évaluation de la diversité génétique de 20 souches d'*A. carbonarius* isolées lors d'une précédente étude.
Méthodologie: Cette étude a porté sur l'évaluation de la diversité génétique de 20 souches d'*A. carbonarius* isolées lors d'une précédente étude. Au total, 26 amorces RAPD ont été initialement testées afin de déterminer les températures d'hybridation optimales et d'identifier celles produisant des profils de bandes clairs, cohérents et reproductibles. Les amorces répondant à ces critères ont ensuite été utilisées pour l'analyse génétique, selon les protocoles RAPD standard.
Résultats: Les résultats obtenus ont révélé que 7 amorces RAPD présentaient des polymorphismes clairs et satisfaisants dans des conditions d'amplification PCR optimales, entre 36°C et 40°C. Ces 7 amorces ont généré 18 loci RAPD distincts à partir des 20 isolats d'*A. carbonarius*. L'analyse de la structure de la population dans la région géopolitique de l'État d'Ogun a révélé que la variation génétique intra-site (de 0,02 à 0,33) représentait la majeure partie de la variation génétique observée (82,0%). L'amplitude de la variation globale due à la divergence au niveau du site (GST) variait de 0,01 à 0,42, suggérant une structure de population minimale au niveau du site. Les relations phylogénétiques entre les 20 isolats d'*A. carbonarius* ont révélé qu'aucun des isolats ne présentait de groupe génétiquement distinct.
Conclusion: La forte variation génétique observée chez les souches d'*A. carbonarius* ochratoxigènes souligne l'importance biotechnologique de la caractérisation moléculaire par RAPD pour la discrimination des souches, l'évaluation de la biosécurité et la gestion ciblée des risques liés à l'ochratoxine A, contribuant ainsi à une sélection et une application éclairée des souches en biotechnologie fongique.

Mots-clés: *Aspergillus carbonarius*; Ochratoxine; Diversité génétique; RAPD

Introduction:

Aspergillus carbonarius is a species of filamentous fungus considered one of the most economically important members of the black Aspergilli due to its frequent occurrence in food, plant, and soil environments (1). The predilection of *A. carbonarius* for different pre- and post-harvest food commodities as well as their known ability for producing ochratoxins including ochratoxin A has been well emphasized (2,3). This ochratoxin A (OTA) which is classified as a group 2B human carcinogen by the International Agency for Cancer Research in 2002, is known to be hepatotoxic, cytotoxic, teratogenic, neurotoxic, mutagenic, and carcinogenic in addition to inhibiting DNA and RNA synthesis (4,5).

Despite the widespread presence of this significant fungal pathogen and its impact on various food products, including ready-to-eat foods (3,6), studies focused on understanding its genetic diversity which is crucial for managing its extensive epidemiological distribution (7) remain limited. Traditionally, phenotypic methods such as the assessment of morphological characteristics, including colony diameter, color, conidial size and texture, and hyphal structure, have been employed (8). Additionally, investigating somatic cell fusion among isolates by screening for vegetative compatibility has also served as a method to examine fungal diversity (9).

These morphological parameters exhibit high variability (10), which limits their resolution and reduces their effectiveness in accurately distinguishing genetic diversity among isolates (11,12) of this fungus. It is therefore important to note that the application of reliable analytical procedures alongside sound quantitative analyses is essential for fungal diversity research (13) to preclude downward bias of genetic variation scores (12). One method that is increasingly gaining recognition is the application of random amplified polymorphic DNA (RAPD) marker analysis because of their documented efficiencies in deciphering the genetic diversities of different biological specimens (14,15). This molecular marker has not only revolutionized and simplified microbial diversity studies (16,17,18), but has also circumvented previous limitations associated with pedigree-based analyses and morphological characterization.

However, despite its advantages such as being cost-effective, rapid, and not requiring prior genomic information, RAPD also presents certain limitations. These include low reproducibility due to sensitivity to reaction conditions, difficulty in distinguishing homologous from non-homologous bands, and challenges in data standardization across laboratories, which can affect the reliability of inter-study comparisons. (18), thereby enhancing the fine resolution required to score appropriate diversity of organisms (12,19). This high throughput technique which is contingent upon amplification of nucleic acid (19,20) is a well understood *in vitro* process (21) with several tools aimed at optimizing their products (22) and thus simplifying the highly complex fungal taxonomy through the determination of the level of polymorphism and simila-

rity amongst fungal strains. This technique has shed light on both the detection of fungal isolates and their genetic relationships (23).

The significant recent advancements in molecular biology tools and technologies have impacted genetic diversity studies (24) and RAPDs, which are random 10-bp primers (decamers) are used in PCR to detect polymorphisms when the DNA sequence is unknown (25). However, there may be variation in the capability of different RAPD markers in the resolution of different fungal isolates. Therefore, the RAPD markers need to be optimized for different fungal isolates. Generally, the RAPD technique is commonly employed in ecological genetics to identify fungal species, estimate genetic patterns of association, analyse composite DNA samples, and develop targeted probe. Therefore, this study aimed to determine the genetic diversity of *A. carbonarius* strains isolated from different sources using the RAPD technique.

Materials and method:

Source of *Aspergillus carbonarius* isolates:

Aspergillus carbonarius isolates utilized in this study were obtained from one of our previous studies (26). The source of the isolates, the code, and the geopolitical zones from which they were isolated in Ogun State, Nigeria, are properly noted in Table 1. The climate of Ogun State is tropically wet and dry with average annual rainfall of 1,340 mm across a total cropland area of 693.21k ha.

All the isolates were obtained between 2018 and 2022 and were appropriately stored at 4°C in a refrigerator (Haier Thermocool, Model HTF-610DM7, Indonesia) in a 50% glycerol stock for future use. These *A. carbonarius* isolates from 'garri' samples, were identified through both phenotypic characterization and molecular analysis, were preserved on Sabouraud Dextrose Agar (SDA) slants and stored at 4 °C under refrigerated conditions for long-term maintenance.

Random amplified polymorphic DNA analysis (RAPD):

Genomic DNA was extracted from the ochratoxigenic *A. carbonarius* isolates using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's recommended protocol. To ensure reproducibility and generate clear, polymorphic DNA banding patterns, RAPD-PCR conditions were systematically optimized. A total of 26 decamer primers (Operon Technologies, USA) were initially screened using the extracted genomic DNA. Each primer was tested under a range of reaction conditions to evaluate its amplification efficiency and reproducibility. Optimization involved systematic variation of key PCR parameters, including primer concentration (0.2–1.0 µM), $MgCl_2$ concentration (1.5–3.0 mM), and template DNA quantity (20–80 ng per reaction). A temperature gradient PCR was used to identify the optimal annealing temperature for each primer, ranging from 36 °C to 40 °C, given the low-stringency binding characteristics of decamer RAPD primers.

Amplification reactions were performed in a total volume of 25 µL, following standard RAPD protocols, with 40 amplification cycles. Primers that consistently produced distinct, reproducible, and polymorphic banding patterns were selected for further analysis. Based on these criteria, 7 primers; OPX07 (GAGCGAGGCT), OPR16 (CTCTGCGCGT), OPR19 (CCTCCTCATC), OPR11 (GTAGCCGTCT), OPV06 (GAACGGACTC), OPA01 (CAGGCCCTTC), and OPA04 (AATCGGG CTG), yielded clear and scorable polymorphic bands. Optimal annealing temperatures for the primers ranged from 36°C to 40°C, with reproducibility confirmed across independent replicates. The final PCR reaction mix (25 µL) contained 12.5 µL of 2×PCR master mix [0.0545 U/µL Taq DNA polymerase in reaction buffer containing 4 mM $MgCl_2$, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP)], 40 pmol of a single decamer primer, and 1 µg of template DNA. The thermal cycling profile was as follows; initial denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 15 sec, annealing starting at 40°C and gradually reduced to 35°C in 4-cycle increments (1 min each), and extension at 72°C for 2 min.

Amplification products were resolved by electrophoresis on 3% agarose gels prepared in 1× TBE buffer, stained with ethidium bromide (0.5 µg/mL), and visualized under UV light using a gel documentation system. Banding patterns were manually analyzed following the criteria of Roodt et al., (27), based on the total number of bands and amplification consistency. Each lane was scored by recording the presence (+) or absence (–) of bands.

All RAPD-PCR reactions were performed in duplicate to assess reproducibility. For each primer and DNA sample, two independent PCR amplifications were carried out under the optimized conditions. Banding patterns were visually compared across replicates, and only bands that were consistently present in all the replicates were scored as true positives. This approach ensured that only reproducible and reliable polymorphic markers were considered for downstream analysis. No numerical averaging of band intensities was performed, as RAPD data

were scored qualitatively based on the presence (1) or absence (0) of bands.

Statistical analysis of data:

All statistical analyses of data were performed using GenAlEx version 6.5. Genetic data were summarized using frequencies, percentages, and diversity indices. RAPD markers were scored as binary data (presence/absence), and allele frequencies were computed for each locus across populations. Genetic diversity parameters (H_S, H_T, and G_{ST}) were estimated following Nei's method. Mean genetic variation was expressed as mean ± SEM.

Analysis of molecular variance (AMOVA) was used to partition genetic variation among and within populations. Chi-square (χ^2) test of independence was used to assess whether the distribution of variable loci and unique genotypes differed significantly among populations from Yewa, Egba, Remo, and Ijebu. Observed frequencies were organized into contingency tables, and expected frequencies were calculated under the null hypothesis of no association between population and genetic category. The degrees of freedom were determined as $(r-1) \times (c-1)$, where r represents the number of populations and c represents the number of categories. Statistical significance was set at $p < 0.05$.

Results:

Seven primers yielded 18 distinct RAPD loci from the studied *A. carbonarius* isolates. The proportion of variable loci observed among the isolates differed across the four populations; Egba (14.3%), Yewa (21.4%), Ijebu (28.6%), and Remo (35.7%) (Table 1). This indicates an increasing trend in genetic variability from Egba to Remo. Similarly, the proportion of unique genotypes identified at these loci also varied among populations, with Egba showing 16.6%, Yewa 22.2%, Ijebu 27.8%, and Remo 33.3% (Table 1). These percentages represent the proportion of genotypes that were exclusive to each population, suggesting increasing genotypic diversity from Egba to Remo. The Simpson's measure of diversity and genotypic evenness for the isolates were all at equilibrium at 0.95 and 1.00, respectively (Table 1).

The allele frequencies of most loci varied significantly ($p<0.05$) among the four populations, with the exception of OPX 07:100 and OPA04:700, which showed no significant variation. Intrapopulation genetic diversity (HS) ranged from 0.02 to 0.33 across loci, while total genetic diversity (HT) ranged from 0.02 to 0.49. The coefficient of genetic differentiation (GST) ranged from 0.01 to 0.42 across all loci (Table 2).

Table 1: Genetic characteristics of *Aspergillus carbonarius* isolates across four regions of Ogun State, Nigeria

Characteristics	**Sample collection sited**			
	YEWA	**EGBA**	**REMO**	**IJEBU**
Number of isolates	5	5	5	5
Number of loci	4	3	6	5
Percentage of variable loci	21.4	14.3	35.7	28.6
Percentage of unique genotypes	22.2	16.6	33.3	27.8
Simpson's Index of Diversity	0.95	0.95	0.95	0.95
Genotype evenness	1.00	1.00	1.00	1.00

Using Chi square test of independence, no significant differences were observed among populations for the proportion of variable loci (χ^2=1.905, df=3, p=0.59) and unique genotypes (χ^2=1.60, df=3, p=0.66)

Table 2: RAPD-based genetic diversity of twenty *Aspergillus carbonarius* isolates from Ogun State, Nigeria

Locus number	**Frequency of the marker allele**				**Genetic Diversity**		
	YEWA=5	**EGBA=5**	**REMO=5**	**IJEBU=5**	**H_S**	**H_T**	**G_{ST}**
OPX 07:100	1.00	1.00	1.00	1.00^{ns}	0.02	0.02	0.03
OPX 07:150	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:200	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:220	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:290	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:310	0.00	0.20	1.00	0.80*	0.16	0.19	0.01
OPX 07:500	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:520	0.80	0.80	0.00	0.00*	0.12	0.20	0.02
OPX 07:700	0.00	0.00	0.00	1.00*	0.11	0.12	0.01
OPX 07:710	0.00	0.00	0.00	1.00*	0.11	0.12	0.01
OPX 07:980	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPX 07:1100	0.20	0.20	1.00	0.80*	0.31	0.34	0.05
OPR16:100	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:150	0.80	0.80	0.80	0.60*	0.12	0.25	0.42
OPR16:200	0.20	0.20	0.20	0.40*	0.32	0.41	0.01
OPR16:210	0.80	0.80	0.80	0.60*	0.12	0.25	0.42
OPR16:220	0.80	0.80	0.80	1.00*	0.12	0.20	0.02
OPR16:290	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:310	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:400	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:450	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:555	0.20	0.20	0.20	0.40*	0.32	0.41	0.01
OPR16:560	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR16:700	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:900	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:1000	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR16:1300	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR16:1600	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR11:270	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:290	0.40	0.20	0.20	0.20*	0.26	0.31	0.07
OPR11:350	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:400	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR11:450	0.80	0.80	0.80	0.60*	0.12	0.20	0.02
OPR11:500	0.20	0.20	0.20	0.40*	0.13	0.24	0.01
OPR19:300	0.20	0.40	0.20	0.60*	0.13	0.24	0.02

OPR19:375	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPR19:500	0.80	0.80	0.20	0.60*	0.12	0.18	0.03
OPR19:800	0.20	0.20	0.20	0.60*	0.13	0.24	0.01
OPR19:900	0.40	0.60	0.20	0.60*	0.19	0.34	0.09
OPV06:500	0.20	0.80	0.20	0.60*	0.33	0.49	0.33
OPV06:550	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPV06:600	0.80	0.20	0.20	0.20*	0.19	0.39	0.13
OPV06:700	0.60	1.00	0.20	0.60*	0.15	0.46	0.17
OPV06:750	0.40	1.00	0.20	0.60*	0.13	0.24	0.01
OPA01:400	0.20	1.00	0.20	0.60*	0.18	0.31	0.18
OPA01:450	0.20	0.60	0.20	0.20*	0.24	0.41	0.05
OPA01:560	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPA01:700	0.20	0.40	0.20	0.60*	0.13	0.24	0.02
OPA01:800	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPA04:380	0.80	0.60	0.20	0.60*	0.21	0.44	0.41
OPA04:390	0.40	0.20	0.20	0.20*	0.26	0.31	0.07
OPA04:700	0.20	0.20	0.20	0.20ns	0.20	0.20	0.03
OPA04:750	0.60	0.60	0.60	0.20*	0.13	0.29	0.44
OPA04:800	0.40	0.80	0.20	0.20*	0.04	0.05	0.09
OPA04:825	0.00	0.00	0.00	0.60*	0.14	0.17	0.01

H_S = intrapopulation genetic diversity; H_T = total genetic diversity; G_{ST}= coefficient of genetic differentiation; ns = not statistically significant; * = statistically significant

Table 3 shows the mean genetic diversity indices of the studied *A. carbonarius* isolates across the four geopolitical zones. The mean intrapopulation genetic diversity (HS) values were 0.28±0.01, 0.21±0.00, 0.27±0.00, and 0.31±0.02 for isolates from Yewa, Egba, Remo, and Ijebu, respectively. The corresponding total genetic diversity (HT) values were 0.32±0.00, 0.28±0.03, 0.36±0.01, and 0.45±0.00.

The proportion of total genetic diversity within populations (HS/HT) was approximately 88%, 75%, 75%, and 69% for Yewa, Egba, Remo, and Ijebu, respectively. The mean GST values were 0.21±0.01 (Yewa), 0.12±0.01 (Egba), 0.17±0.01 (Remo), and 0.23±0.00 (Ijebu).

Table 3: Mean genetic variation of the studied *Aspergillus carbonarius* isolates

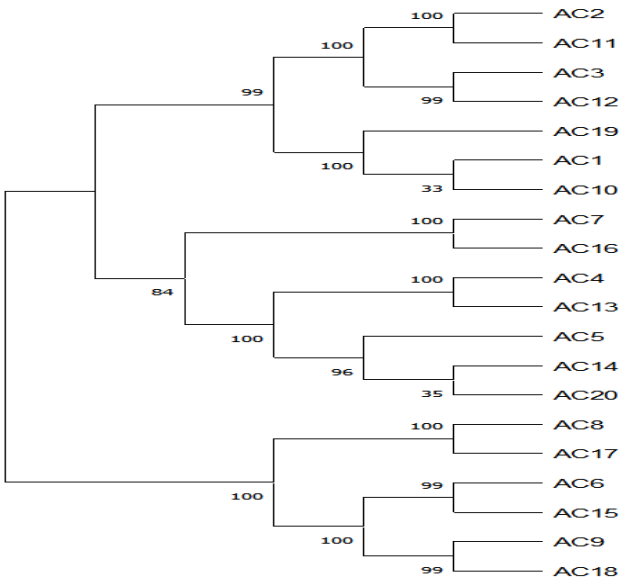
GEPZ/OS	**Number of loci**	**Mean ± SEM**		
		H_S	**H_T**	**G_{ST}**
YEWA	22	0.28 ± 0.01	0.32 ± 0.00	0.21 ± 0.01
EGBA	23	0.21 ± 0.00	0.28 ± 0.03	0.12 ± 0.01
REMO	35	0.27 ± 0.00	0.36 ± 0.01	0.17 ± 0.01
IJEBU	30	0.31 ± 0.02	0.45 ± 0.00	0.23 ± 0.00

GEPZ/OS=Geopolitical Zones of Ogun State, SEM=Standard Error of Mean

Table 4: Analysis of Molecular Variance (AMOVA) for the loci of *Aspergillus carbonarius* isolates

Sources of variation	df	SS	MS	F
Between-treatments	3	10.2727	3.4242	0.81178
Within-treatments	40	168.7273	4.2182	
Total 43 179				

F-ratio of 0.81178; *p*-value of 0.494904; result not statistically significant at $p<0.05$



AC1-AC5 = Yewa isolates, AC6-10 = Egba isolates, AC11-15 = Remo isolates, AC16-AC20 = Ijebu Isolates

Fig 1: Phylogenetic characterization of ochratoxigenic *Aspergillus carbonarius*

The analysis of molecular variance validated that even though the genetic diversity of isolates within samples from specific geopolitical zones accounted for majority of the total genetic diversity, the observations when compared between different zones were not statistically significant at $p<0.05$ (Table 4).

The phylogenetic relationships among the 20 *A. carbonarius* isolates are depicted in Fig 1. None of the isolates formed location-designated genetic clusters, while 3 of the 5 isolates from the Yewa zone formed a cluster with 99% similarity, while the remaining 2 Yewa isolates grouped with isolates from Egba, Remo, and Ijebu, showing 84% similarity. Similarly, 3 of the 5 isolates from Egba formed clusters with Remo and Ijebu at a 100% level of similarity, but both Remo and Ijebu are interspersed by isolates sharing different levels of similarities with isolates from all the geopolitical zones (84-100%).

Discussion:

The importance of RAPD in deciphering the genetic characterization of living organisms has been well documented (28,29,30). In this study, the strains of ochratoxigenic *A. carbonarius* are between 14.3% to 35.7% of variable loci, 16.6 to 33.3% of unique genotypes, Simpson's diversity index of 0.95 and genotypic evenness of 1 to highlights extensive genetic diversity within and between the isolates (7).

The genotypic evenness of 1 could further delineate that the population of the isolates yielded equally abundant genotypes. The observed values for total gene diversity (HT), within-subpopulation diversity (HS), and genetic differentiation among subpopulations (GST) are consistent with those reported for fungi possessing known sexual reproduction cycles. This is because several studies have documented that fungi

that reproduce through sexual means demonstrate wider genetic diversity than those with asexual means of reproduction (7,31,32). In another vein, it has been reported that some fungal isolates known to reproduce asexually have a genetic diversity index higher than expected, while the importance of parasexuality of some fungi in influencing diversity under controlled field conditions has also been emphasized (7).

Genotypic diversity analysis, nested analysis of molecular variance, and phylogenetic analysis consistently indicated that majority of the genetic differences within the organisms is attributable to differences across unique isolates. These analyses also revealed significant differentiation among isolates from each of the geopolitical zones, and such spatial community arrangement is possible through gene flow (33, 34). The occurrence of this type of gene flow within and between geographic populations of *A. carbonarius* can be accomplished by wind-aided spore dispersal, sexual and asexual reproduction (34). Most of the RAPD allele frequencies of the studied *A. carbonarius* except for two strains, shows apparent variation within the studied populations, to further connote that there might be frequent interbreeding events among these strains of *A. carbonarius* (35)

The comparatively low genetic divergence among the studied isolates (GST) suggests that genetic material (alleles) is being exchanged between and within populations across a broad spatial range, resulting in increased genetic similarity. There may be a need to do more intensive sampling of *A. carbonarius* at the geopolitical zones to examine if the aforementioned inter-collection sites variation reflects the actual disparities affecting how populations exchanged genetic material.

The fact that none of the isolates formed site-specific genetic clusters is an indication that allele frequencies among the studied *A. carbonarius* populations are very similar (34). It is thus imperative to note that any efforts aimed at controlling the ochratoxigenic *A. carbonarius* should take into account their extensive genetic diversities as well as factors prompting such high levels of diversity.

Conclusion:

In conclusion, the detection of high genetic variation among ochratoxigenic *A. carbonarius* strains emphasizes the biotechnological relevance of RAPD marker analysis as a rapid and effective tool for strain differentiation. In biotechnology, such genetic information is critical for biosafety evaluation, the identification of highly toxigenic genotypes, and the selection of appropriate strains for industrial, agricultural, or environmental applications. The findings, therefore, provide a molecular framework that supports informed decision-making in fungal biotechnology and mycotoxin risk management.

Contributions of authors:

BTT and ODP were involved in the study conceptualization and design; BTT, MOC, OST, KEP, ANT, GMA and ODP were involved in the methodology and data analysis; BTT, TOA, and SLO wrote the original draft; BTT, MOC, OAB and ODP reviewed and edited the manuscript. The final version of the manuscript was reviewed and approved by all authors.

Source of funding:

No funding was received for the study.

Conflict of interest:

No conflict of interest is declared.

References:

1. Yu, J., Cleveland, T. E., Nierman, W. C., and Bennett, J. W. *Aspergillus flavus* genomics: gateway to human and animal health, food safety, and crop resistance to diseases. Rev Iberoam Micol. 2005; 22 (4): 194–202. https://doi.org/10.1016/S1130-1406(05)70043-7
2. Sahab, A. F., Aly, S., Hathout, A. S., Ziedan, E. S., and Sabry, B. A. Application of some plant essential oils to control *Fusarium* isolates associated with freshly harvested maize in Egypt. J Essent Oil Bear Plants. 2014; 17 (6): 1146–1155. https://doi.org/10.1080/0972060X.2014.891447
3. Thomas, B. T., and Ogunkanmi, L. A. Ochratoxin A producing filamentous fungi in 'garri' circulating in Ogun State, Nigeria. Elixir Biosci. 2014; 75: 27788–27794.
4. Samuel, M. S., Jeyaram, K., Datta, S., Chandrasekar, N., Balaji, R., and Selvarajan, E. Detection, contamination, toxicity, and prevention methods of ochratoxins: An updated review. J Agric Food Chem. 2021; 69 (46): 13974–13989. https://doi.org/10.1021/acs.jafc.1c05994
5. Thomas, B. T., Popoola, O. D., Efuntoye, M. O., Coker, M. O., and Tajudeen, A. O. Pathological scanning of ochratoxigenic moulds impaired feed in vivo, towards conceptualizing their reverberations on different organs. Tanzania J Sci. 2021; 47 (2): 625–636. https://doi.org/10.4314/tjs.v47i2.18
6. Gil-Serna, J., García-Díaz, M., Vázquez, C., González-Jaén, M. T., and Patiño, B. Significance of *Aspergillus niger* aggregate species as contaminants of food products in Spain regarding their occurrence and their ability to produce mycotoxins. Food Microbiol. 2019; 82: 240–248. https://doi.org/10.1016/j.fm.2019.02.013
7. Pekarek, E., Jacobson, K., and Donovan, A. High levels of genetic variation exist in *Aspergillus niger* populations infecting *Welwitschia mirabilis* Hook. J Hered. 2006; 97 (3): 270–278. https://doi.org/10.1093/jhered/esj031
8. Salim, R. G., Aly, S. E., Abo-Sereh, N. A., Hathout, A. S., and Sabry, B. A. Molecular identification and inter-simple sequence repeat (ISSR) differentiation

of toxigenic *Aspergillus* strains. Jordan J Biol Sci. 2019; 12 (5): 609-616.
9. Leslie, J. F. Fungal vegetative compatibility. Annu Rev Phytopathol. 1993; 31 (1): 127–150.
10. Kumeda, Y., and Asao, T. Heteroduplex panel analysis, a novel method for genetic identification of *Aspergillus* section *Flavi* strains. Appl Environ Microbiol. 2001; 67 (9): 4084–4090. https://doi.org/10.1128/AEM.67.9.4084-4090.2001
11. Ogunkanmi, L. A., Ogundipe, O. T., and Fatokun, C. A. Molecular characterization of cultivated cowpea (*Vigna unguiculata* L. Walp) using simple sequence repeat markers. Afr J Biotechnol. 2014; 13 (34): 3464-3472. https://doi.org/10.5897/ajb2013.13166
12. Thomas, B. T., Ogunkanmi, L. A., Iwalokun, B. A., and Agu, G. C. Molecular characterization and strain typing of fungal contaminants of processed *Manihot esculenta Crantz* ("garri") in Ogun State, Nigeria. Ann Health Res. 2017; 3 (2): 112–117.
13. May, R. M. Patterns of species abundance and diversity. In: Cody, M. L., and Diamond, J. M. (eds). Ecology and Evolution of Communities. Cambridge (MA): Belknap Press of Harvard Univ Press; 1979: 81–120.
14. Mazumder, S. R., Hoque, H., Sinha, B., Chowdhury, W. R., Hasan, M. N., and Prodhan, S. H. Genetic variability analysis of partially salt tolerant local and inbred rice (*Oryza sativa* L.) through molecular markers. Heliyon. 2020; 6 (8): e04333. https://doi.org/10.1016/j.heliyon.2020.e04333
15. Niklas, A., and Olszewska, D. Application of the RAPD technique to identify genetic diversity in cultivated forms of *Capsicum annuum* L. Biotechnologia. 2021; 102 (2): 209-223. https://doi.org/10.5114/bta.2021.106523
16. Garcia, A. A., Benchimol, L. L., Barbosa, A. M., Geraldi, I. O., Souza Jr., C. L., and Souza, A. P. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet Mol Biol. 2004; 27: 579–588. https://doi.org/10.1590/S1415-47572004000400019
17. Yuste-Lisbona, F. J., Capel, C., Gómez-Guillamón, M. L., Capel, J., López-Sesé, A. I., and Lozano, R. Codominant PCR-based markers and candidate genes for powdery mildew resistance in melon (*Cucumis melo* L.). Theor Appl Genet. 2011; 122: 747–758. https://doi.org/10.1007/s00122-010-1483-6
18. Laursen, M. F., Bahl, M. I., Michaelsen, K. F., and Licht, T. R. First foods and gut microbes. Front Microbiol. 2017; 8: 356. https://doi.org/10.3389/fmicb.2017.00356
19. Amusa, O. D., Ogunkanmi, L. A., Adetunbi, J. A., Akinyosoye, S. T., Bolarinwa, K. A., and Ogundipe, O. T. Assessment of bruchid (*Callosobruchus maculatus*) tolerance of some elite cowpea (*Vigna unguiculata*) varieties. J Agric Sustain. 2014; 6 (2): 164–178.
20. Zhang, H. T., Kacharmina, J. E., Miyashiro, K., Greene, M. I., and Eberwine, J. Protein quantification from complex protein mixtures using a proteomics methodology with single-cell resolution. Proc Natl Acad Sci U S A. 2001; 98 (10): 5497–5502.https://doi.org/10.1073/pnas.101124598
21. Mullis, K. B. The polymerase chain reaction. New York: Springer Sci Bus Media; 1994; 41 (5): 1-427.
22. Rozen, S., and Skaletsky, H. Primer3 on the www for general users and for biologist programmers. In: Bioinformatics Methods and Protocols. 1999: 365–386. https://doi.org/10.1385/1-59259-192-2:365
23. Paplomatas, E. J. Molecular diagnostics for soilborne fungal pathogens. Phytopathol Mediterr. 2004; 43 (2): 213–220. https://digital.casalini.it/10.1400/14591
24. Frankham, R. Introduction to conservation genetics. Cambridge: Cambridge University Press; 2002.
25. Abdelmigid, H. M. Efficiency of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity studies in canola. Afr J Biotechnol. 2012; 11 (24): 6409–6419. https://doi.org/10.5897/AJB11.127
26. Thomas, B. T. Biodiversity and control of ochratoxigenic moulds isolated from cassava flakes. Ph.D. Thesis. University of Lagos, Akoka, Lagos, Nigeria; 2018.
27. Roodt, R., Spies, J. J., and Burger, T. H. Preliminary DNA fingerprinting of the turfgrass *Cynodon dactylon* (Poaceae: Chloridoideae). Bothalia. 2002; 32: 117–122. https://doi.org/10.4102/abc.v32i1.474
28. Mei, Z., Zhang, C., Khan, M. A., et al. Efficiency of improved RAPD and ISSR markers in assessing genetic diversity and relationships in *Angelica sinensis* (Oliv.) Diels varieties of China. Electron J Biotechnol. 2015; 18 (2): 96–102. https://doi.org/10.1016/j.ejbt.2014.12.006
29. Titilayo, O. A., Samuel, A. O., David, O. O., and Adewunmi, T. I. Genetic variations among three major ethnic groups in Nigeria using RAPD. Mol Biol Res Commun. 2018; 7 (2): 51-58. https://doi.org/10.22099/mbrc.2018.26098.1280
30. Hromadová, Z., Gálová, Z., Mikolášová, L., Balážová, Ž., Vivodík, M., and Chňapek, M. Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean. Plants (Basel). 2023; 12 (15): 2763. https://doi.org/10.3390/plants12152763
31. Gosselin, L., Jobidon, R., and Bernier, L. Genetic variability and structure of Canadian populations of *Chondrostereum purpureum*, a potential biophytocide. Mol Ecol. 1999; 8: 113–122. https://doi.org/10.1046/j.1365-294X.1999.00543.x
32. Johannesson, H., Gustafsson, M., and Stenlid, J. Local population structure of the wood decay ascomycete *Daldinia loculata*. Mycologia. 2001; 93: 440–446. https://doi.org/10.1080/00275514.2001.12063176
33. Rydholm, C., Szakacs, G., and Lutzoni, F. Low genetic variation and no detectable population structure in *Aspergillus fumigatus* compared to closely related *Neosartorya* species. Eukaryotic Cell. 2006; 5 (4): 650–657. https://doi.org/10.1128/ec.5.4.650-657.2006
34. Klaassen, C. H., Gibbons, J. G., Fedorova, N. D., Meis, J. F., and Rokas, A. Evidence for genetic differentiation and variable recombination rates among Dutch populations of the opportunistic human pathogen *Aspergillus fumigatus*. Mol Ecol. 2012; 21 (1): 57–70. https://doi.org/10.1111/j.1365-294X.2011.05364.x
35. Gryta, H., Debaud, J. C., and Marmeisse, R. Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. Heredity (Edinb). 2000; 84: 294–302. https://doi.org/10.1046/j.1365-2540.2000.00668.x

Bamidele et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 339 - 349 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2492. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

SARS-CoV-2 faecal negativity and gastrointestinal eubiosis in non-severe COVID-19 in Lagos, Nigeria

*Bamidele, T. A., Fowora, M. A., Raheem, T. Y., Ajayi, A., Adagbada, A., Yisau, J. I., Salau, O. Y., Shaibu, J. O., Amoo, O. S., Aiyedogbon, N., Fesobi, T. W., Akintunde, G., Iwalokun, B. A., Smith, S. I., and Salako, B. L.

Molecular Biology and Biotechnology Department, Nigerian Institute of Medical Research, 6, Edmund Crescent, Yaba-Lagos, Nigeria
*Correspondence to: bamideletj@gmail.com; Tel: +2348038578093; ORCID: https://orcid.org/0000-0003-2155-8639

Abstract:

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), when disseminated to the gastrointestinal tract (GIT), can cause alterations in the composition and diversity of the gastrointestinal tract microbiota. However, there is a paucity of data linking SARS-CoV-2 fecal negativity with the GI microbial balance. This study investigated the association between the GI bacterial composition and clinically defined asymptomatic, mild/moderate COVID-19 fecal-negative individuals.
Methodology: This was a cross sectional, comparative study of GI bacterial composition in COVID-19 nasopharyngeal (NP) -positive (n=7) and negative (n=5) participants at the testing facility of the Nigerian Institute of Medical Research between 6th July and 8th August 2022. Faecal samples were collected from the 12 participants. The RNA extracted from the NP-positive samples was used for RT–qPCR detection of the nucleocapsid and open reading frame (*ORF*1ab) genes, while DNA from all the faecal samples was used for 16S rRNA metataxonomic analysis.
Results: The participants comprised males (n=4) and females (n=8), ages 17-74 years. The NP-positive participants reported no (n=2, 28.5%), mild (n=4, 57.1%) and moderate (n=1, 14.3%) clinical symptoms. The viral genes were undetected with uniform or rich bacterial species in the fecal samples. The most abundant bacterial phyla were the Firmicutes, Bacteroidota, and Proteobacteria while *Prevotella copri, Phocaeicola vulgatus*, and the immuno-modulatory, anti-inflammatory bacterium, *Faecalibacterium prausnitzii*, were the dominant species. There were no significant differences in alpha diversity, Pielou evenness (p=0.223) and Shannon richness index (p=0.062), or beta taxonomic diversity (PERMANOVA p=0.357) between NP-positive and negative participants.
Conclusion: This study did not reveal any differences in the gut bacterial community between asymptomatic and mild/moderate COVID-19 patients, and apparently healthy controls. The asymptomatic, mild, and moderate COVID-19 patients in this study maintained balanced gut bacterial diversity, with undetected viral gene in their stool, which may pose little or no threats to the public health in terms of SARS-CoV-2 shedding to the environment. This may however not be generalizable given the small sample size.

Keywords: Gastrointestinal, eubiosis, microbiome, SARS-CoV-2, fecal, RT–PCR.

Received Feb 4, 2026; Revised Mar 17, 2026; Accepted Mar 18, 2026

Négativité fécale du SRAS-CoV-2 et eubiose gastro-intestinale dans le cas du COVID-19 non sévère à Lagos, Nigeria

*Bamidele, T. A., Fowora, M. A., Raheem, T. Y., Ajayi, A., Adagbada, A., Yisau, J. I., Salau, O. Y., Shaibu, J. O., Amoo, O. S., Aiyedogbon, N., Fesobi, T. W., Akintunde, G., Iwalokun, B. A., Smith, S. I., et Salako, B. L.

Département de Biologie Moléculaire et de Biotechnologie, Institut Nigérian de Recherche Médicale, 6, Edmund Crescent, Yaba-Lagos, Nigeria
*Correspondance à: bamideletj@gmail.com; Tél: +2348038578093; ORCID: https://orcid.org/0000-0003-2155-8639

Résumé:

Contexte: Le coronavirus 2 du syndrome respiratoire aigu sévère (SARS-CoV-2), lorsqu'il se dissémine dans le tractus gastro-intestinal (TGI), peut entraîner des altérations de la composition et de la diversité du microbiote intestinal. Cependant, les données liant la négativité fécale au SARS-CoV-2 à l'équilibre microbien gastro-intestinal sont rares. Cette étude a examiné l'association entre la composition bactérienne gastro-intestinale et les individus asymptomatiques, présentant une forme légère à modérée de COVID-19 et dont les selles sont négatives.
Méthodologie: Il s'agit d'une étude transversale comparative de la composition bactérienne gastro-intestinale chez des participants positifs (n=7) et négatifs (n=5) au test nasopharyngé (NP) de dépistage de la COVID-19, recrutés au centre de dépistage de l'Institut nigérian de recherche médicale entre le 6 juillet et le 8 août 2022. Des échantillons de selles ont été prélevés chez les 12 participants. L'ARN extrait des échantillons NP positifs a été utilisé pour la détection par RT-qPCR des gènes de la nucléocapside et du cadre de lecture ouvert (*ORF*1ab), tandis que l'ADN de tous les échantillons de selles a été utilisé pour l'analyse métataxonomique de l'ARNr 16S.
Résultats: Les participants étaient composés de 4 hommes et 8 femmes, âgés de 17 à 74 ans. Les participants NP positifs ont rapporté des symptômes cliniques absents (n=2, 28,5%), légers (n=4, 57,1%) et modérés (n=1, 14,3%). Les gènes viraux étaient indétectables, malgré une composition bactérienne homogène ou abondante dans les échantillons fécaux. Les phyla bactériens les plus abondants étaient les Firmicutes, les Bacteroidota et les Proteobacteria, tandis que *Prevotella copri*, *Phocaeicola vulgatus* et la bactérie immunomodulatrice et anti-inflammatoire *Faecalibacterium prausnitzii* étaient les espèces dominantes. Aucune différence significative n'a été observée concernant la diversité alpha, l'équitabilité de Pielou (p=0,223), l'indice de richesse de Shannon (p=0,062) ou la diversité taxonomique bêta (PERMANOVA p=0,357) entre les participants positifs et négatifs au test NP.
Conclusion: Cette étude n'a révélé aucune différence dans la composition du microbiote intestinal entre les patients asymptomatiques et ceux atteints d'une forme légère à modérée de COVID-19, et les sujets témoins apparemment sains. Les patients atteints de COVID-19 asymptomatiques, légères ou modérées inclus dans cette étude ont conservé une diversité bactérienne intestinale équilibrée, sans détection de gène viral dans leurs selles. Ceci suggère que le risque de propagation du SARS-CoV-2 dans l'environnement pourrait être faible, voire nul. Toutefois, ces résultats ne sont pas généralisables compte tenu de la petite taille de l'échantillon.

Mots-clés: Système gastro-intestinal, eubiose, microbiome, SARS-CoV-2, selles, RT-PCR.

Introduction:

The 2019 coronavirus disease (COVID-19) pandemic resulted in an unprecedented global crisis; a large number of disease-associated deaths occurred on different scales in countries across the world. This prompted research directed towards both pharmaceutical and non-pharmaceutical interventions, while testing was also encouraged to increase. The Nigerian Institute of Medical Research, Nigeria, initiated a modified drive-through testing platform where the largest number of patients were tested in the country (1,2). Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the aetiology of the disease (COVID-19), is transmitted through respiratory droplets, aerosols, and sometimes the faeco-oral route (3).

Despite the primary involvement of the upper respiratory tract, substantial viral infection has been reported in the gastrointestinal tract (GIT) of patients with moderate to severe COVID-19 and viral stool shedding even weeks after disease resolution (4-9). However, little or no viral stool clearance has been reported in patients with mild to moderate disease, which comprises up to 81% of those who contract COVID-19 (10,11).

The pathophysiology of SARS-CoV-2 and its link with the GIT have been well reported. After contact, the virus binds to angiotensin-converting enzyme 2 (ACE-2) receptors in the lungs. The viral spike glycoprotein attached to ACE-2 enables viral entry into cells, which leads to replication and dissemination to other parts of the body. The intestinal epithelium, which highly expresses ACE-2 receptors, can harbor SARS-CoV-2 after swallowing viral-laden sputum (gut-lung cross talk); this serves as a site of extrapulmonary viral infection, as evidenced by the presence of SARS-CoV-2 RNA in the feces of patients with COVID-19 (7,12-15).

The role of the gut microbiota in the development of many diseases, including immune-mediated, metabolic, neurodegenerative, and psychiatric diseases, is becoming increasingly clear (16-18). An imbalance in the intestinal microbial community/diversity that can be associated with disease is referred to as dysbiosis. The most typical characteristics are a depletion in the richness and diversity of the microbiota, a loss of probiotics/beneficial microbiota or an overgrowth of harmful ones.

Additionally, host-specific factors such as lifestyle habits, hygiene, infections, inflammation, high sugar intake, low fiber intake, and drugs can cause dysbiosis (19-21). Several human observational studies have reported dysbiosis of the gut microbiota composition in COVID-19 patients at the time of diagnosis and during the course of the disease, with decreased bacterial diversity observed in COVID-19 patients (13,22,23). These studies documented cases in populations in China, the USA, Japan,

Bangladesh, the UAE, Portugal, Germany, and Hungary. In Africa and Nigeria, there is a paucity of data connecting the gut microbiome with mild and asymptomatic COVID-19. Many other studies have linked the composition of the gut microbiota to severe COVID-19 and GI symptoms. Therefore, this study was designed to investigate the diversity of the gut microbiota and extent of viral shedding in patients with clinically defined asymptomatic, mild, or moderate COVID-19.

Materials and method:

Study site:

This study was carried out on drive-in and walk-through COVID-19 testing facilities at the Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria. The NIMR is a leading medical research institute located in the commercial capital (Lagos) of Nigeria. Lagos is the most populous city in Nigeria, with approximately 24 million inhabitants (https://lagosstate.gov.ng/about-lagos/). During the COVID-19 pandemic, the COVID-19 testing facilities in NIMR received more than 50,000 people, making it one of the largest in the country.

Ethical considerations:

The conduct of this study was approved (IRB/21/069) by the institutional review board of the Nigerian Institute of Medical Research (NIMR-IRB), while social approval was given by the Lagos State Ministry of Health. The written informed consent obtained from the participants after they understood the research and are locked up in a cabinet that is only accessible to the Principal Investigator. The electronic copies of all data (questionnaire and result sheets) are anonymized using unique codes and saved on a passworded computer.

Study design:

This was a cross-sectional comparative study of SARS-CoV-2-infected patients and apparently healthy Nigerians (negative control) in Lagos. For the purpose of this study, N refers to nasopharyngeal (NP) negative while P refers to nasopharyngeal (NP) positive participants.

Sample size calculation:

Using a two-sided t-test, a significance level (α) of 0.05, and assuming an effect size (Cohen's d) of 0.8 (a large effect size), the required sample size for 80% power was estimated to be 26 participants. However, our study included less than half of this required number, this implies a key limitation of our study, highlighting the need for future research with a larger sample size to validate our findings and improve the robustness of statistical inferences.

Data capture:

Sociodemographic data of participants, including hospital admission history, symptoms, presence/absence of comorbidities, and treatment/drug intake, were collected via a short questionnaire administered by the research assistant after consent form was signed.

Sample collection and nucleic acid extraction:

Stool samples were collected from the consenting participants visiting the NIMR walk-in and drive-through testing facilities following standard procedures. The selection of participants was not in a particular format but rather at convenience. They were recruited into the study as they visited the facilities, irrespective of their demographics. Stool samples were collected from 6th July 2022 till 8th August 2022 in an OMNIgene GUT microbiome device (DNA Genotech Inc., Ottawa, Canada) and stored in a freezer until processing.

Viral RNA extraction was performed on 10th August 2022 on all samples from participants who tested positive for nasopharyngeal RT–qPCR (NP) using Qiagen kits (QIAamp Viral RNA, Germany), while total DNA was extracted from all stool samples on 12th of August 2022 using ZymoBiomics DNA Miniprep metagenomics kits (Zymo Research, USA). All the nucleic acid extraction was done according to the manu facturer's instructions. The extracts were quantitated, and the purity was evaluated using a Nanodrop One/One spectrophotometer (Thermo Scientific, UK).

Gene amplification and metagenomic analysis:

The viral nucleocapsid (*N*) and open reading frame (*ORF*1ab) genes were detected on 10th of August 2022 by RT–qPCR (Applied Biosystems) using the SARS–CoV-2 Detection Assay (SCODA) workflow. Briefly, 5µl of extracted RNA was added to 20µl of Mastermix to make a 25µl reaction mixture with the viral synthetic genes and RNase-free water as positive and negative controls, respectively. The PCR conditions included a cycle of reverse transcription at 52°C for 10 min; inactivation at 95°C for 10 seconds; 40 cycles of denaturation at 95°C for 5 seconds; and annealing and extension at 56°C for 30 seconds. The cutoff cycle threshold for the target genes was 38.

Amplification of the V4 region was carried out using the 16S V4 updated primer sequence 515F/806R, forward barcoded: FWD: GTGYCAGCMGCCGCGGTAA; REV: GGACTACNV GGGTWTCTAAT (24,25), in a 25µl reaction mixture containing 0.8X PCR master mix (Platinum Hot Start PCR Master Mix (2x)), 0.2 µM of each

primer, 1µl of template DNA, and PCR grade water (Sigma). The thermocycling condition included an initial denaturation at 94°C for 3 min, followed by 35 amplification cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s. This was followed by a final extension cycle at 72°C for 10 min. All the samples were amplified in triplicate and pooled into single 75µl volumes.

Amplicons were tested on a Bioanalyzer to confirm the presence of the PCR product. The amplicons were also run on a 1.5% agarose gel, and a single band of approximately 360 bp was used to confirm successful amplification. Subsequently, the amplicons were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher/Invitrogen) following the manufacturer's instructions.

The pooled amplicons were cleaned using the MoBio UltraClean PCR Clean-Up Kit following the manufacturer's instructions. The purity and concentration of the PCR products were subsequently measured. Samples with an A260/A280 ratio between 1.8 and 2.0 were aliquoted and used for library preparation and metagenomics sequencing (Laragen, Inc., USA).

Metagenomic sequencing:

The pure library was pooled and denatured into single strand, further diluted to concentration suitable for loading on the P1 flow cell of the Illumina NextSeq 2000 sequencing instrument with 15% PhiX control spike-in. The raw data (FASTQ files) were analyzed and annotated on One Codex database involving k-mer based classification, artifact filtering and species-level abundance estimation.

Taxonomic classification and data analysis:

Alpha diversity of the microbial community in the two groups of participants (positive-P, negative-N) was assessed by Pielou and Shannon indices using Kruskal–Walli's test of significance. The Pielou diversity index was used to determine the uniformity of the microbial community, while the Shannon index was used to determine the richness of the microbial community.

Beta taxonomic diversity was assessed with the Bray–Curtis' diversity index using permutational multivariate analysis of variance (PERMANOVA) for the test of significance and principal component analysis calculator (26) was used to determine variation in the microbiota of the participants. The taxonomic classification was performed using the Greengenes2 database trained for hypervariable 4 of the 16S rRNA (gg_2022_10_backbone.v4.nb).

Results:

The participants comprised males (n=4) and females (n=8), ages 17-74years. The NP-positive participants reported no (n=2, 28.5%), mild (n=4, 57.1%) and moderate (n=1, 14.3%) clinical symptoms. Of the 12 participants whose stool samples were analyzed, 7 presented with various degrees of symptoms; asymptomatic (n=2), mild (n=4) or moderate (n=1), while the others (n=5) were negative for NP-related COVID-19 from RT–PCR assay (Table 1).

Table 1: Symptomatology and demographics of COVID-19 study participants

Study ID	Symptoms	Sex	Age (years)
16-P	Asymptomatic	F	74
17-P	Asymptomatic	F	37
12-P	Mild	F	27
31-P	Mild	F	27
4-P	Mild	F	17
ME-P	Mild	F	58
3-P	Moderate	F	44
7-N	Negative	M	38
8-N	Negative	M	31
13-N	Negative	F	36
20-N	Negative	M	28
24-N	Negative	M	22

M = Male; F = Female; ID = Identification

Table 2: Bray–Curtis distance matrix of the samples

Sample	**Species similarity**											
	12-P	**13-N**	**16-P**	**17-P**	**20-N**	**24-N**	**3-P**	**31-P**	**4-P**	**7-N**	**8-N**	**ME-P**
12-P	0	0.8814	0.7842	0.8395	0.8379	0.742	0.7435	0.7087	0.8831	0.8072	0.7548	0.7589
13-N	0.8814	0	0.7893	0.9595	0.9579	0.8998	0.7269	0.7736	0.7436	0.889	0.9013	0.8953
16-P	0.7842	0.7893	0	0.8107	0.909	0.758	0.7041	0.7171	0.7861	0.8272	0.8586	0.8195
17-P	0.8395	0.9595	0.8107	0	0.5215	0.7554	0.7463	0.8003	0.9177	0.6325	0.8562	0.859
20-N	0.8379	0.9579	0.909	0.5215	0	0.8501	0.7759	0.8734	0.9554	0.4443	0.8	0.8729
24-N	0.742	0.8998	0.758	0.7554	0.8501	0	0.8317	0.6636	0.881	0.8201	0.8353	0.5227
3-P	0.7435	0.7269	0.7041	0.7463	0.7759	0.8317	0	0.7101	0.6378	0.6371	0.9075	0.8414
31-P	0.7087	0.7736	0.7171	0.8003	0.8734	0.6636	0.7101	0	0.6573	0.7651	0.873	0.7548
4-P	0.8831	0.7436	0.7861	0.9177	0.9554	0.881	0.6378	0.6573	0	0.8304	0.9357	0.9324
7-N	0.8072	0.889	0.8272	0.6325	0.4443	0.8201	0.6371	0.7651	0.8304	0	0.8234	0.8317
8-N	0.7548	0.9013	0.8586	0.8562	0.8	0.8353	0.9075	0.873	0.9357	0.8234	0	0.8712
ME-P	0.7589	0.8953	0.8195	0.859	0.8729	0.5227	0.8414	0.7548	0.9324	0.8317	0.8712	0

Values from 0 to 1 indicate identical composition to distinct species, where 0 represents similarity. The higher the figure, the greater the distance and dissimilarity

SARS-CoV-2 RNA was not detected in any stool sample by RT–qPCR. Sequencing of the 16S rRNA v4 amplicon yielded 747,917 sequences for all the analyzed samples. After quality control, 585,356 sequences belonging to 806 features were used for further analysis. The Pielou diversity did not significantly differ between the P and N groups (Kruska-Wallis p= 0.22). Similarly, the Shannon index was not significantly different between the two groups (Kruska-Wallis p=0.062).

The Bray–Curtis beta diversity was also not significantly different between the two groups (PERMANOVA p=0.357) and this is depicted in Table 2. However, the bacterial community of the microbiota of some of the participants who tested positive and negative for COVID-19 showed close clustering (3-P, 4-P, 16-P, and 13-N; 7-N, 20-N, and 17-P) and high divergence, as in the case of sample 8-N, as depicted in Fig 1. Sample ME-P, which was positive, had a bacterial community that clustered with and overlapped that of sample 24-N, which was negative for COVID-19 (Fig 2).

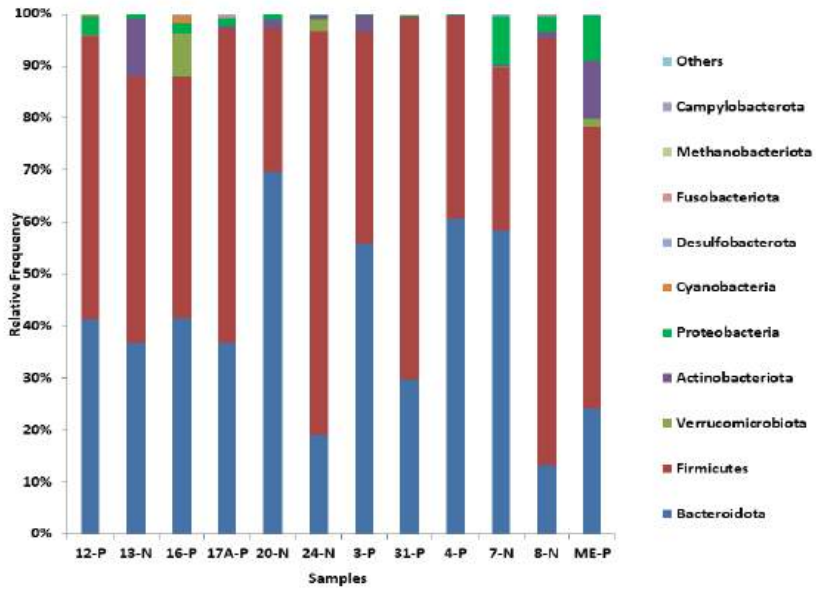


Fig 1: Relative abundance of bacterial phyla in the gut microbiota of COVID-19 positive and negative participants

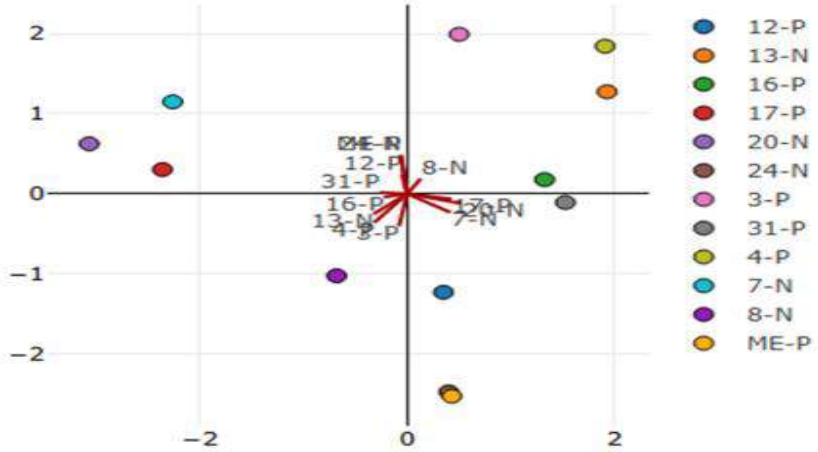


Fig 2: Principal Component Analysis (PCA) of the bacterial species in the gut microbiota of COVID-19 positive and negative participants.

Ten major phyla were identified among others in the 12 samples. Firmicutes and Bacteriodota were the most abundant phyla in the samples, with relative abundances of 82.2%, 54.6%, 46.3%, and 27.7% (Firmicutes) and 13.2%, 41.2%, and 41.7% 69.6% (Bacteriodota) in samples 8 N, 12-P, 16-P, and 20-N, respectively, as shown in Fig 1. At the genus level, four genera, namely, *Faecalibacterium*, *Phocaeicola*, *Prevotella*, and *Bacteroides*, were the most dominant. The genus *Phocaeicola* was more dominant in samples 16-P and 4-P, with relative abundances of 15.9% and 19.2%, respectively. *Faecalibacterium* was more dominant in sample 8-N, with a relative abundance of 47.7%.

Akkermansia was only present in samples 12-P, 16-P, 24-N, 7-N, and ME-P, with relative abundances of 0.03%, 8.0%, 2.2%, 0.1% and 1.2%, respectively (Fig 3). *Prevotella copri*, *Phocaeicola vulgatus,* and *Faecalibacterium prausnitzii* were the most dominant among the other species identified. Although *Faecalibacterium prausnitzii* was the dominant species identified, this bacterium was not detected in the 13 N subgroup. Similarly, *Prevotella copri* was absent in 12-P, 13-N, and 16-P. Among the other identified dominant species, *Bacteroides stercoris* (23.1%) and *Gemmiger qucibialis* (16.7%) were more dominant in sample 4 (Fig 4).

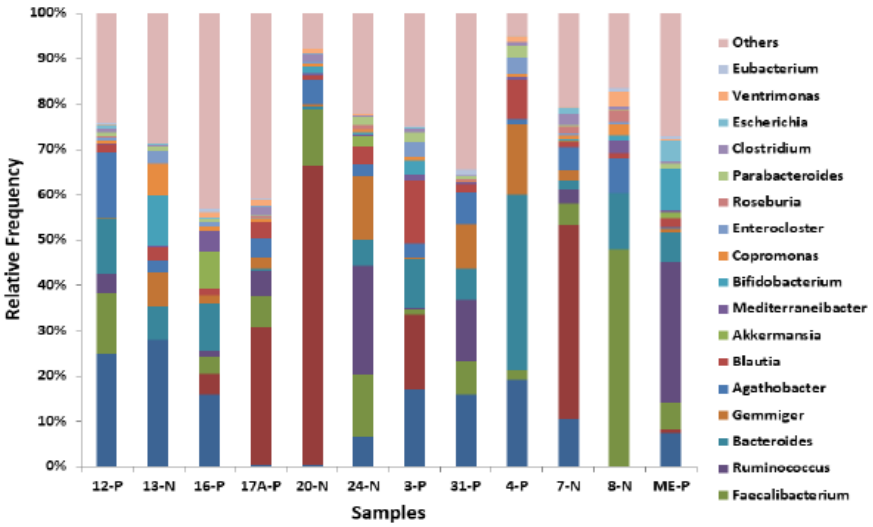


Fig 3: Relative abundance of bacteria genera in the gut microbiota of COVID-19 positive and negative participants.

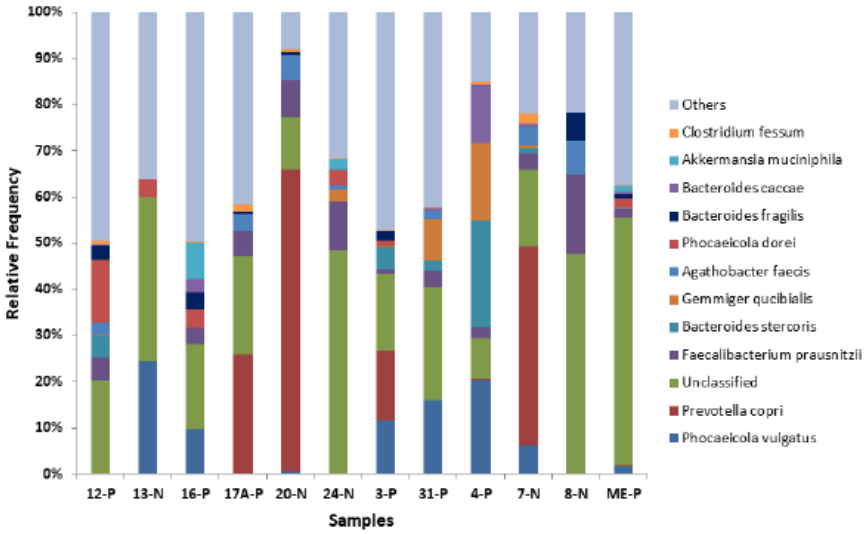


Fig 4: Relative abundance of bacteria species in the gut microbiota of COVID-19 positive and negative participants.

Discussion:

The asymptomatic, mild and moderate COVID-19 patients in this study maintained balanced gut bacterial diversity, with undetected viral genes in their stool. This poses no threats to the public health in terms of SARS-CoV-2 shedding to the environment, although this may not be generalizable given the small sample size. The small sample size is attributed to the transient nature of the disease as at the time the samples were being collected. The visit to the testing platform was already waning and as such, we set out to recruit as many as would give written informed consent without minding the sex, age group. The symptoms were extracted from their self-completed online questionnaire and as confirmed after clinical examination at the site by medical doctors. Many of the participants just wanted to confirm their status and were not ready to provide any stool samples while a few others who collected the sample containers gave the excuse of distance from the facility.

There have been conflicting reports on intestinal microbial diversity in patients with COVID-19; a lower intestinal diversity has been reported in these patients than in healthy controls in China and Bangladesh respectively (27,28), while a different pattern was demonstrated in another study in a Christian hospital, Hong Kong (29). It has also been reported in studies from China that higher intestinal microbial richness is observed in asymptomatic patients than in symptomatic patients and during disease progression (30-32). Although we were unable to collect severe or hospitalized cases in the present study, intestinal microbial evenness (p=0.22) and richness (p=0.06) were not associated with asymptomatic, mild, or moderate cases compared to COVID-19 negative control. In contrast, Kim et al., (33) reported intestinal dysbiosis after infection in patients with asymptomatic or mild COVID-19 in Korea.

The classification of asymptomatic or mild COVID-19 was performed according to the severity criteria of the Korea Centers for Disease Control and Prevention (http://ncov.mohw.go.ks/en/); age younger than 60 years, no underlying chronic diseases, an alert mentality, a body temperature less than 37.5°C, and no radiological evidence of pneumonia. In our study, the World Health Organization (WHO) severity criteria, which are slightly different, were used (34). These findings may be attributed to the fact that SARS-CoV-2 has not crossed the gut-lung axis of infected participants at the time of sample collection or because host immunity has taken care of it. This finding supports the report that severe cases are associated with a disruption of gut microbial diversity/richness. This is corroborated by the lack of detection of viral RNA in the stool samples of all NP-positive study participants.

The viability of the nucleic acid (viral RNA and metagenomic DNA) in the stool collection device (OMNIgene GUT) was preserved, as it was stored frozen at -80°C. This needs to be mentioned given the 'unstable' nature of RNA which is delicate to handle compared to DNA. Also, the device is formulated to preserve nucleic acid (DNA at room temperature and RNA at ultra-low temperature of -80°C).

Taxonomically, bacteria are classified according to seven levels; kingdom, phylum, class, order, family, genus, and species. However, only a few phyla are represented, and they account for more than 160 species (35). The phyla Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia are dominant in the healthy gut, with Firmicutes and Bacteroidetes representing 90% of the gut microbiota (36). This was also observed in our study, in which more than 90% of the samples were from one of the 2 phyla. Additionally, in our study, Campylobacterota, Methanobacteriota, Desulfobacterota, and Cyanobacteria were present in some of the samples, while the others were almost invisible. Within the Firmicutes in this study, the obligately anaerobic, Gram positive, spore-forming *Clostridium fessum* was only present in the 7- N, 4-P, 17A-P, and 12-P. This bacterium was isolated for the first time in 2021 by Seo et al., (37) from the faeces of a healthy Korean subject.

The balance in microbial diversity in the gut of the study participants is in tandem with the findings of some reports (29,38-41), although some were in mice (40,41), the microbial species that negatively correlate with COVID-19 severity, such as the anti-inflammatory agent, immune modulatory bacteria, *Faecalibacterium prausnitzii*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis* and *Eubacterium rectale,* were richly abundant, as reported by other studies (29,38). Notably, all participants were predominantly infected with *Prevotella copri* in the intestinal tract, regardless of the status of COVID-19. This bacterium, prevalent in non-Westernized populations, has been shown to act as a 'double edged sword' in health and disease (39-41).

This study has some limitations. Although the small sample size (n=12) is a limitation, which was due to the transient nature of the pandemic as at the time of this study, our data are similar to those of Yeoh et al., (29), Kim et al., (33), and Mesoraca et al., (42). The

study however offers preliminary evidence that may inform future investigations into the relationship between SARS-CoV-2 faecal negativity and gastrointestinal eubiosis in non-severe COVID-19 cases. Also, the sex disparity observed in this study was due to the fact that more females were reporting for testing than the males. There seems to be discordant reports on the association of sex, age and geographic origin with gut microbiota in two studies (43, 44), conducted among donors from different regions including France, Denmark, Germany, Italy, Netherlands, UK and Sweden, although these studies employed fluorescence in-situ hybridization and flow cytometry in their microbiota analysis.

Conclusion:

The gut microbial diversity in asymptomatic, mild or moderate COVID-19 individuals in this study was not disrupted with beneficial bacteria such as *Prevotella copri*, *Phocaeicola vulgatus,* and *Faecalibacterium prausnitzii* being predominant. The viral RNA was undetected in their stool samples and therefore would not be able to shed the pathogen to the environment through the stool.

Acknowledgments:

The funding of this study by the COVID-19 Intervention Fund of the Nigerian Institute of Medical Research is well acknowledged. Additionally, Mrs. Sasonel Adebiyi of the Department of Molecular Biology and Biotechnology of the National Institute of Medical Research (NIMR) helped in typing the manuscript, Ms. Esther Momoh assisted in running the stool RT–qPCR, and Dr. Chika Onwuamah provided the Qiagen RNA extraction kits. The assistance of the technical staff at the Human Virology and Genomics of the Nigerian Institute of Medical Research (NIMR), where the stool and nasopharyngeal RT–qPCR analyses were performed, is well appreciated.

Contributions of authors:

TAB conceptualized the study; TAB, MAF, TYR, and BAI designed the study; TAB, MAF, TYR, AbA, OYS, OSA, JOS, NA, TWF, CJI, BAI, SIS, and BLS were involved in sample collection, processing and data analysis; and TAB, MAF, TYR, AbA, AjA, JIY, GA, OYS, OSA, BAI, SIS, and BLS contributed to the writing of the manuscript. All the authors approved the final manuscript submitted for publication.

Source of funding:

This study was supported by the COVID-19 Intervention Funds of NIMR (grant # NMG-CIF-21-0069-2) which provided funds for only collection and analysis of the data.

Conflict of interests:

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Data availability:

The datasets used and/or analyzed during the study are available from the corresponding author (TAB) on reasonable request.

Disclaimer:

The views expressed in this article are the authors' and not an official position of the institution or funder. The authors are responsible for the article results, findings, and content.

References:

1. Amoo, O. S., Ohihoin, A. G., Musa, A. Z., et al. Implementation of a modified drive-through sampling strategy for SARS-CoV-2-the Nigerian experience. Pan Afr Med J. 2020; 35(2): 107. https://doi:10.11604/pamj.supp.2020.35.2.24319
2. Ohihoin, A. G., Osuolale, K. A., Okwuraiwe, P. A., et al. Outcome of Symptom-Based RT–PCR Testing for SARS-CoV-2: Experience from a Large Public Testing Centre in Nigeria. Adv Infect Dis. 2022; 12 (4): 799-812. https://doi.org/10.4236/aid.2022.124057
3. Harrison, A. G., Lin, T., and Wang, P. Mechanisms of SARS-CoV-2 transmission and pathogenesis. Trends Immunol. 2020; 41(12):1100–1115. https:// doi: 10.1016/j.it.2020.10.004.
4. Lamers, M. M., Beumer, J., van der Vaart, J., et al. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020; 369(6499): 50-54. doi: 10.1126/science.abc1669.
5. Zang, R., Castro, M. F. G., McCune, B., et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol. 2020: 5(47): eabc3582. doi: 10.1126/sciimmunol.abc3582.
6. Zuo, T., Zhang, F., Lui, G. C. Y., et al. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. Gastroenterol. 2020; 159 (3): 944-955. doi: 10.1053/j.gastro.2020.05.048
7. Xiao, F., Tang, M., Zheng, X., et al. Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterol. 2020; 158 (6): 1831–1833. doi: 10.1053/j.gastro.2020.02.055
8. Natarajan, A., Zlitni, S., Brooks, E. F., et al. Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. Med. 2020; 3(6): 371–387. doi: 10.1016/j.medj.2022.04.001.

9. Zollner, A., Koch, R., Jukic, A., et al. Post-acute COVID-19 is characterized by gut viral antigen persistence in inflammatory bowel diseases. Gastroenterol. 2022: 163(2): 495–506. doi: 10.1053/j.gastro.2022.04.037.
10. Wu, Z., and McGoogan, J. M. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China. J Am Med Assoc. 2020; 323(13): 1239-1242. https://doi:org/10.1001/jama.2020.2648.
11. Oran, D. P., and Topol, E. J. The Proportion of SARS-CoV-2 Infections That Are Asymptomatic: A Systematic Review. Ann Intern Med. 2021; 174 (5):655–662. https://doi:10.7326/M20-6976.
12. Shang, J., Wan, Y., Luo, C., et al. Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci USA. 2020; 117(21):11727–11734. doi: 10.1073/pnas.2003138117
13. Guo, W., Li, M., Dong, Y., et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev. 2020; 36(7): e3319. https://doi:10.1002/dmrr.3319.
14. Zhang, D., Li, S., Wang, N., Tan, H. Y., Zhang, Z., and Feng, Y. The cross-talk between gut microbiota and lungs in common lung diseases. Front Microbiol. 2020; 11: 301. doi: 10.3389/fmicb.2020.00301.
15. Gupta, A., and Madhavan, M. V. Extrapulmonary manifestations of COVID-19. Nat Med. 2020; 26(7):1017–1032. https://doi:10.1038/s41591-020-0968-3.
16. Feigin, V. L., Vos, T., Alahdab, F., et al. Burden of Neurological Disorders Across the US From 1990–2017: A Global Burden of Disease Study. JAMA Neurol. 2021; 78(2): 165–176. doi: 10.1001/jamaneurol.2020.4152.
17. Fan, Y., and Pedersen, O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021; 19: 55–71. doi: 10.1038/s41579-020-0433-9
18. Hrncir, T., Hrncirova, L., Kverka, M., and Tlaskalova-Hogenova, H. The role of gut microbiota in intestinal and liver diseases. Lab Anim. 2019; 53 (3): 271–280. doi: 10.1177/0023677218818605.
19. Van der Merwe, M., Moore, D., Hill, J. L., et al. The Impact of a Dried Fruit and Vegetable Supplement and Fiber Rich Shake on Gut and Health Parameters in Female Healthcare Workers: A Placebo-Controlled, Double-Blind, Randomized Clinical Trial. Microorganisms. 2021; 9(4): 843. doi: 10.3390/microorganisms9040843.
20. Shahinozzaman, M., Raychaudhuri, S., Fan, S., and Obanda, D. N. Kale Attenuates Inflammation and Modulates Gut Microbial Composition and Function in C57BL/6J Mice with Diet-Induced Obesity. Microorganisms. 2021; 9(2): 238. doi: 10.3390/microorganisms9020238.
21. Kohnert, E., Kreutz, C., Binder, N., et al. Changes in Gut Microbiota after a Four-Week Intervention with Vegan vs. Meat-Rich Diets in Healthy Participants: A Randomized Controlled Trial. Microorganisms. 2021; 9(4): 727. doi: 10.3390/microorganisms9040727.
22. Gaibani, P., D'Amico, F., Bartoletti, M., et al. The gut microbiota of critically ill patients with COVID-19. Front Cell Infect Microbiol. 2021; 11:670424. doi: 10.3389/fcimb.2021.670424.
23. Xu, R., Lu, R., Zhang, T., et al. Temporal association between human upper respiratory and gut bacterial microbiomes during the course of COVID-19 in adults. Commun Biol. 2021; 4: 240. doi: 10.1038/s42003-021-01796-w
24. Parada, A. E., Needham, D. M., and Fuhrman, J. A. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2015; 18 (5): 1403-1414. http://dx.doi.org/10.1111/1462-2920.13023.
25. Apprill, A., McNally, S., Parsons, R., and Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015; 75: 129–137. http://dx.doi.org/10.3354/ame01753.
26. Principal Component Analysis (PCA) Calculator-Scree plot, 3D PCA, 2D PCA, 1D PCA. https://www.statskingdom.com/pca-calculator.html. (Accessed 25/7/2024)
27. Ren, Z., Wang, H., Cui, G., et al. Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. Gut. 2021; 70(7): 1253-1265. https://doi:10.1136/gutjnl-2020-323826.
28. Chen, R. Y., Mostafa, I., Hibberd, M. C., et al. A microbiota-directed food intervention for undernourished children. N Engl J Med. 2021; 384 (16): 1517–1528. doi: 10.1056/NEJMoa2023294.
29. Yeoh, Y. K., Zuo, T., Lui, G. C., et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut. 2021; 70 (4): 698-706. https://doi:10.1136/gutjnl-2020-323020.
30. Zou, L., Ruan, F., Huang, M., et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med. 2020; 382(12):1177–1179. doi: 10.1056/NEJMc2001737
31. Liu, X., Li, Y., Wu, K., Shi, Y., and Chen, M. Fecal Microbiota Transplantation as Therapy for Treatment of Active Ulcerative Colitis: A Systematic Review and Meta-Analysis. Gastroenterol Res Pract. 2021; https://doi:10.1155/2021/6612970.
32. Lin, R., Xiao, M., Cao, S., et al. Distinct gut microbiota and health outcomes in asymptomatic infection, viral nucleic acid test re-positive, and convalescent COVID-19 cases. MLife. 2022; 1(2): 183-197. doi: 10.1002/mlf2.12022.
33. Kim, H. N., Joo, E. J., Lee, C. W., et al. Reversion of gut microbiota during the recovery phase in patients with asymptomatic or mild covid-19: longitudinal study. Microorganisms. 2021; 9 (6): 1237. https://doi:10.3390/microorganisms9061237.
34. World Health Organization. Clinical management of COVID-19: Interim Guidance. WHO. 2020; 13-15.
35. Laterza, L., Rizzatti, G., Gaetani, E., Chiusolo, P., and Gasbarrini, A. The gut microbiota and immune system relationship in human graft-versus-host disease. Mediterr J Haematol Infect Dis. 2016; 8(1): e2016025. doi: 10.4084/MJHID.2016.025.
36. Arumugam, M., Raes, J., Pelletier, E., et al. Enterotypes of the human gut microbiome. Nature. 2011; 473: 174–180. doi: 10.1038/nature09944
37. Seo, B., Jeon, K., Baek, I., Lee, Y. M., Baek, K., and Ko, G. P. *Clostridium fessum* sp. nov., isolated from human feces. Int J Syst Evol Microbiol. 2021; 71(1): 004579. https://doi:10.1099/ijsem.0.004579.
38. Schult, D., Reitmeier, S., Koyumdzhieva, P., et al. Gut bacterial dysbiosis and instability is associated with the onset of complications and mortality in COVID-19. Gut Microbes. 2022; 14 (1): 2031840. https://doi:10.1080/19490976.2022.2031840.
39. Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., et al. Human gut microbes impact host serum metabo- lome and insulin sensitivity. Nature. 2016; 535:376–381. doi: 10.1038/nature18646
40. De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., Duchampt, A., Bäckhed, F., and Mithieux, G. Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metab. 2016; 24(1):151–157. doi: 10.1016/j.cmet.2016.06.013.
41. Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., et al. Dietary fiber-induced improvement in glucose

metabolism is associated with increased abundance of Prevotella. Cell Metab. 2015; 22 (6):971–982. doi: 10.1016/j.cmet.2015.10.001

42. Mesoraca, A., Margiotti, K., Viola, A., Cima, A., Sparacino, D., and Giorlandino, C. Evaluation of SARS-CoV-2 viral RNA in fecal samples. Virol J. 2020; 17(1): 86. https://doi.org/10.1186/s12985-020-01359-1.
43. Lay, C., Rigottier-Gois, L., Holmstrom, K., et al. Colonic microbiota signatures across five Norther European countries. Appl Environ Microbiol. 2005; 71(7): 4153-4155. doi: 10.1128/AEM.71.7.4153-4155.2005
44. Mueller, S., Saunier, K., Hanisch, C., et al. Differences in fecal microbiota in different European study populations in relation to age, gender and country: a cross-sectional study. Appl Environ Microbiol. 2006; 72 (2):1027-1033. doi: 10.1128/AEM.72.2.1027-1033.2006.

Sangodare et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 350 - 360 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2488. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Preservative potentials and stability of bacteriocin-producing lactic acid bacteria of dairy origins

[1]Sangodare, A. O., *[2]Omotoso, I. O., [1]Omotoso, O. G., and [3]Samuel, T. A.

[1]Department of Science Laboratory Technology, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[2]Department of Environmental Health Science, Faculty of Basic Medical Science, Ajayi Crowther University, Oyo, Oyo State, Nigeria
[3]Department of Microbiology, Faculty of Life Science, University of Ilorin, Ilorin, Nigeria
*Correspondence to: omotosoifeoluwa@gmail.com; +2348068193274

Abstract:

Background: Lactic acid bacteria (LAB) from dairy sources are known to produce bacteriocins with promising antimicrobial properties. The variations in their preservative effectiveness and stability under different environmental conditions create a gap in knowledge. This study aimed to produce bacteriocin and determine their stability properties of bacteriocin isolated from lactic acid bacteria indigenous to selected fermented diary (cheese and 'Nunu') within Ilorin metropolis.
Methodology: Isolation and identification of bacteriocin-producing lactic acid bacteria (LAB) from fermented dairy products and bacteriocin purification, were carried out by standard methods. Bacteriocin stability tests were performed and characterized by heat resistance, sensitivity to pH and proteolytic enzyme by the method described by Gautam and Sharma. Bio-preservation efficacy of bacteriocin was evaluated through microbial load determination of the food products after treatment with purified bacteriocin, with and without refrigeration. Molecular identification of the bacteriocin-producing LAB was determined by 16S rRNA sequencing.
Results: Four LAB were isolated from the food products and phenotypically identified as *Lactobacillus* spp and *Streptococcus* spp. The bacteriocin-producing LAB were identified by 16s RNA sequencing as *Lactobacillus fermentum* strain TSGB1241, *Lactococcus fermentum* strain DMF18, *Lactococcus lactis* subsp *cremoris* and *Pediococcus pentosaceus* SL4. The antibacterial activity of bacteriocin produced by the LAB was affected by an increase temperature and maximum activity was recorded at acidic pH (2.0-4.0), which indicates its potential use in acidic food. The antibacterial activity was completely lost during treatment with proteolytic enzyme (pepsin). The preservative test showed the potential of refrigerated bacteriocin to reduce microbial load that may contaminate stored food products.
Conclusion: Bacteriocin produced from cheese and 'Nunu' offer bio-preservative efficacy and provide stability to intrinsic and extrinsic factors that can affect food spoilage. This bacteriocin offers natural and safe and effective preservation to foods products.

Keywords: Fermented diary; Lactic acid bacteria; preservatives; Stability; Bacteriocin

Received Jan 19, 2026; Revised Mar 20, 2026; Accepted Mar 22, 2026

Potentiel de conservation et stabilité des bactéries lactiques productrices de bactériocines d'origine laitière

[1]Sangodare, A. O., *[2]Omotoso, I. O., [1]Omotoso, O. G., et [3]Samuel, T. A.

[1]Département de Technologie des Laboratoires Scientifiques, Faculté des Sciences Pures et Appliquées, Université de Technologie Ladoke Akintola, Ogbomoso, État d'Oyo, Nigéria
[2]Département des Sciences de la Santé Environnementale, Faculté des Sciences Médicales Fondamentales, Université Ajayi Crowther, Oyo, État d'Oyo, Nigéria

[3]Département de Microbiologie, Faculté des Sciences de la Vie, Université d'Ilorin, Ilorin, Nigéria
*Correspondance à: omotosoifeoluwa@gmail.com; +2348068193274

Résumé:

Contexte: Les bactéries lactiques (BAL) d'origine laitière sont connues pour produire des bactériocines aux propriétés antimicrobiennes prometteuses. Les variations de leur efficacité et de leur stabilité en tant que conservateurs dans différentes conditions environnementales créent un manque de connaissances. Cette étude visait à produire des bactériocines et à déterminer leurs propriétés de stabilité. Ces bactériocines ont été isolées de bactéries lactiques indigènes de certains produits laitiers fermentés (fromage et «Nunu») de la métropole d'Ilorin.
Méthodologie: L'isolement et l'identification des bactéries lactiques (BAL) productrices de bactériocines à partir de produits laitiers fermentés, ainsi que la purification des bactériocines, ont été réalisés selon des méthodes standard. Des tests de stabilité des bactériocines ont été effectués et caractérisés par leur thermorésistance, leur sensibilité au pH et leur activité enzymatique protéolytique, selon la méthode décrite par Gautam et Sharma. L'efficacité de la bactériocine en tant que biopréservatrice a été évaluée par la détermination de la charge microbienne des produits alimentaires après traitement avec la bactériocine purifiée, avec et sans réfrigération. L'identification moléculaire des BAL productrices de bactériocines a été déterminée par séquençage de l'ARNr 16S.
Résultats: Quatre bactéries lactiques (BAL) ont été isolées des produits alimentaires et identifiées phénotypiquement comme appartenant aux genres *Lactobacillus* et *Streptococcus*. Les BAL productrices de bactériocine ont été identifiées par séquençage de l'ARN 16S comme étant *Lactobacillus fermentum* souche TSGB1241, *Lactococcus fermentum* souche DMF18, *Lactococcus lactis* subsp. *cremoris* et *Pediococcus pentosaceus* SL4. L'activité antibactérienne de la bactériocine produite par les BAL était influencée par l'augmentation de la température et une activité maximale a été observée à pH acide (2,0-4,0), ce qui indique son potentiel d'utilisation dans les aliments acides. L'activité antibactérienne était totalement abolie lors d'un traitement à la pepsine. Le test de conservation a démontré le potentiel de la bactériocine réfrigérée pour réduire la charge microbienne susceptible de contaminer les produits alimentaires stockés.
Conclusion: La bactériocine produite à partir de fromage et de «Nunu» présente une efficacité de biopréservation et assure une stabilité face aux facteurs intrinsèques et extrinsèques susceptibles d'altérer les aliments. Cette bactériocine offre une conservation naturelle, sûre et efficace des produits alimentaires.

Mots-clés: Produits laitiers fermentés; Bactéries lactiques; Conservateurs; Stabilité; Bactériocine

Introduction:

Antimicrobial peptides (AMPs) are important component that serve as one of the earliest defense mechanisms against invading pathogens (1). These peptides exhibit strong potential as antimicrobial against a wide range of microorganisms, which make them a promising option for the development of novel therapeutic agents. In contrast to most conventional antibiotics, AMPs commonly act by disrupting microbial cell membranes, forming transmembrane pores, and in some cases enhancing host immune responses through immunomodulatory functions (2).

Among AMPs, bacteriocin are the most studied group. Their preservative potentials as antimicrobial largely depends on their ability to associate with and destabilize bacterial cell membranes or cell walls. Typically, AMPs possess an overall positive charge and a high proportion of hydrophobic amino acids, which enables selective attraction to the negatively charged surfaces of bacterial membranes. Once bound, AMPs disrupt membrane integrity through non-enzymatic mechanisms (3).

A major advantage of AMPs is their selective toxicity, as effective antimicrobial peptides must exhibit minimal harm to host mammalian cells. Structural and functional differences between microbial and mammalian membranes including lipid composition, membrane potential, polarity, and organization and contribute to this selectivity and influence the diverse mechanisms of action observed among AMP classes (4). Also, several AMPs demonstrate diverse antimicrobial and immunological activities, suggesting their importance as tools in biomedical applications.

Beyond their direct antimicrobial effects, AMPs can modulate immune responses, which further enhances their therapeutic potential. These peptides offer multiple advantages over traditional antibiotics, including broad-spectrum activity, low propensity for resistance development, synergistic effects with existing antibiotics, and the ability to neutralize endotoxins and regulate immune function (5). AMPs can also interfere with various intracellular processes such as cell wall synthesis, protein production, and nucleic acid replication (6) as well as inhibit formation of bacterial biofilms (7). Additionally, their immunomodulatory roles allow them to regulate inflammation and mitigate septic responses during infection.

Lactic acid bacteria (LAB) are group of Gram-positive, non-spore-forming rods, cocci, and cocco-bacilli, that produces lactic acid as a major product while making use of carbohydrates during fermentation process. LAB are lar-

gely employed for the processing of many fermented food products and beverages. It is well known that LAB, which contribute more to the properties of foods, mediate the fermentation process and may also exhibit antibacterial activity against clinical pathogens. These bacteria not only produce lactic acid as an end product of carbohydrate catabolism but can also produce organic substances that contribute to the flavor and aroma; aiding unique organoleptic characteristics to the products (8). LAB exopolysaccharide (EPS) is economically important because it can impart functional effect to foods and confer beneficial health effects to the consumer.

Lactic acid bacteria are normal, physiological flora of the mouth, intestines and female genital tract. The *Lactobacillus* genus is one of the most important genera in the group of LAB. *Lactobacillus* strains are Gram-positive, catalase-negative, non-spore-forming and non-motile bacilli (9). Traditional and molecular techniques have successfully been used to divide bacteria belonging to the LAB family into the following genera; *Lactobacillus, Streptococcus, Lactococcus, Enterococcus, Melissococcus, Tetragenococcus, Vagococcus, Trichococcus, Carnobacterium, Desemzia, Leuconostoc, Weisella* and *Pediococcus* (10). Gram-positive bacteria belonging to the phylum Actinobacteria, such as the genera *Aerococcus, Microbacterium, Propionibacterium* and *Bifidobacterium* also produce lactic acid.

The antibacterial peptides/bacteriocin produced by *Bacillus safensis* strain MK-12 isolated from waste dump soil have been characterized and optimized by Sajid et al., (11). These bacterial isolates were screened for antagonistic activity against a set of American Type Culture Collections (ATCC) and local multi-drug-resistant human pathogenic bacterial strains and discovered that the soil were reservoir of these strains exhibiting antibacterial activities and could be a promising source of antimicrobial compounds to tackle against resistant bacteria in future. Therefore, this study aimed to identify bacteriocin from locally selected fermented diary ('Nunu' and cheese) and evaluate their stability and bio-preservative efficacies against some selected drug-resistant pathogens and food spoilage bacteria.

Materials and method:

Study setting:

The study is conducted in the Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. The study area is located at approximately latitude 8.479 9° N and longitude 4.5418° E within the University.

Collection and isolation of Lactic Acid Bacteria from selected dairy samples:

Fermented dairy products ('Nunu' and cheese) were purchased at the University kiosk, in the early hour of the day, transported in ice-bag and preserved in the refrigerator before microbial analysis. Samples were serially diluted in sterile distilled water up to 10^{-5} folds, and 0.5ml from 10^{-3} and 10^{-5}dilution factors were plated on MRS agar using the pour plating method. The plates were then incubated anaerobically in air-tight anaerobic jar at 37^0C for 48 hours. Colonies were randomly picked and sub-cultured on MRS agar and incubated anaerobically to obtain pure culture.

Preliminary screening for the identification of the lactic acid bacteria:

The isolated lactic acid bacteria were screened by their microscopic, morphological and biochemical features carried out according to the descriptions in the Bergey's Manual of Determinative Bacteriology (12).

Tests organisms*:*

The test bacteria used to evaluate the microbial effects of the LAB include *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* ATC 25922, methicillin-sensitive *Staphylococcus aureus* 25923, *Salmonella* Typhi*, Enterococcus faecalis, Bacillus megaterium, Serratia marcescens, Citrobacter freundii* and *Staphylococcus aureus* ATCC 25913, which were obtained from the Department of Microbiology, University of Ilorin, Nigeria.

Stability tests for LAB producing bacteriocin:

Bacteriocin from the LAB was purified by precipitation of the bacteria supernatants in ammonium sulphate followed by dialysis to remove excess salt. The stability test of the bacteriocin for heat resistance, sensitivity to pH and proteolytic enzymes was performed according to the method described by Gautam and Sharma (13).

Heat resistance:

Aliquots (5ml) of partially purified bacteriocin were heated at 50^0C, 75^0C, 100^0C in a water bath and at 121^0C in an autoclave for 15 minutes. The treated samples were tested for antibacterial activity against the indicator bacterial strains.

pH sensitivity:

Two and a half (2.5ml) ml aliquots of

the partially purified bacteriocin was taken in a sample bottle and the pH of the contents were adjusted to pH 2, 4, 7 and 9 separately, using either diluted NaOH or HCl (1M NaOH solution). After allowing the samples to stand at room temperature for 2 hours, the antimicrobial activity was assayed according to Fugaban et al., (14).

***Sensitivity to proteolytic enzymes**:*

The LAB supernatant fluid was treated for 1 hour at 37^0C in Pepsin (Sigma) at final concentration of 1 mg/ml. The mixtures were heated in boiling water for 3 minutes to denature the enzymes, before testing the bacteriocin activity.

Bio-preservation potential bacteriocin for food products

Enumeration and isolation of microorganisms at different preservation method:

The microbial loads of the food products (meat and cheese) obtained fresh from the University of Ilorin kiosk were evaluated by four different forms of treatment at 24 hours interval; (i) samples of food products were trea ted with 5% bacteriocin (obtained by diluting 5 ml of the purified bacteriocin in 100 ml of distilled water); (ii) samples were treated with 5% bacteriocin and refrigerated; (iii) samples of the food products were refrigerated only; and (iv) samples of food products had no form of treatment. Enumeration and isolation of the microbial load were carried out at an interval of 24 hours.

Determination of microbial load:

The microbial load of the food products at 24 hours interval on every stage and treatment was carried out by the serial dilution of the food samples from 10^{-1} to 10^{-6} using 1ml of meat and cheese samples for the various treatment and 200µl of samples plated unto nutrient agar plates and incubated at 37°C for 24 hours. The numbers of colonies in duplicate were counted using a digital colony counter to determine the mean (±SD) microbial loads in the sample. The microbial loads of bacteriocin-treated samples were compared with non-treated control samples.

***Characterization of bacterial isolates by 16S rRNA sequencing**:*

Further characterization of the isolates by molecular techniques for better taxonomical data was done using 16s rRNA amplification and Sanger's sequencing, followed by bioinformatic analysis and phylogenetic tree construction. Using the sequence alignment generated, the phylogenetic tree was constructed using the neighbor joining method while maximum composite likelihood method was used to compute evolutionary distance with the bioinformatics software Mega X (15).

Data analysis:

Results were analyzed by analysis of variance (ANOVA) followed by Dunnett's *t*-test. The Statistical Package for the Social Sciences (SPSS, version 26.0) was used to determine the mean and standard deviation of the microbial loads for each sample and probability was accepted at $p \leq 0.05$ significance.

Results:

Isolation and identification of lactic acid bacteria (LAB):

A total of four LAB were isolated based on their morphological characteristics from the 'Nunu' and cheese samples. They were Gram-positive, rod and cocci shaped. The microscopic, morphological and biochemical features of the LAB isolates are presented in Table 1.

Table 1: Microscopic morphology and biochemical characteristics of isolated Lactic Acid Bacteria

Isolates	Gram staining	Morphology	Glucose	Sucrose	Fructose	Dextrose	Maltose	Lactose	Spore Formation	Indole	Methyl Red	Citrate	Catalase	Starch hydrolysis	Oxidase	Oxygen relationship	TSI			Probable identity
																	1	2	3	
M1	+ve	Rod, cream, circular dull, entire	+/+	+/+	+/-	+/-	+/-	+/+	-	-	-	+	-	+	-	FA	L, G, S	-	A	*Lactobacillus* sp
M2	+ve	Rod, cream, irregular, dull, undulate	+/-	+/-	+/-	+/-	+/-	+/+	-	-	-	-	-	-	-	FA	L	-	A	*Lactobacillus* sp
M3	+ve	Cocci, cream, circular, dull, undulate	+/+	+/+	-/-	+/-	+/-	+/+	-	-	-	+	-	-	-	FA	L, G, S	-	A	*Streptococcus*
M4	+ve	Cocci, white, circular, glistering, entire	+/+	+/-	+/-	+/-	+/-	+/+	-	-	-	-	-	+	-	FA	L & G	-	A	*Streptococcus*

TSI = triple sugar iron test (1- gas production, 2- H_2S production, 3- glucose-sucrose and lactose), +/+ = Acid and Gas producer**;** +/- = Acid producer only**;** -/- = No Acid and Gas produced**;** A= Acid producer only**; -** =Negative

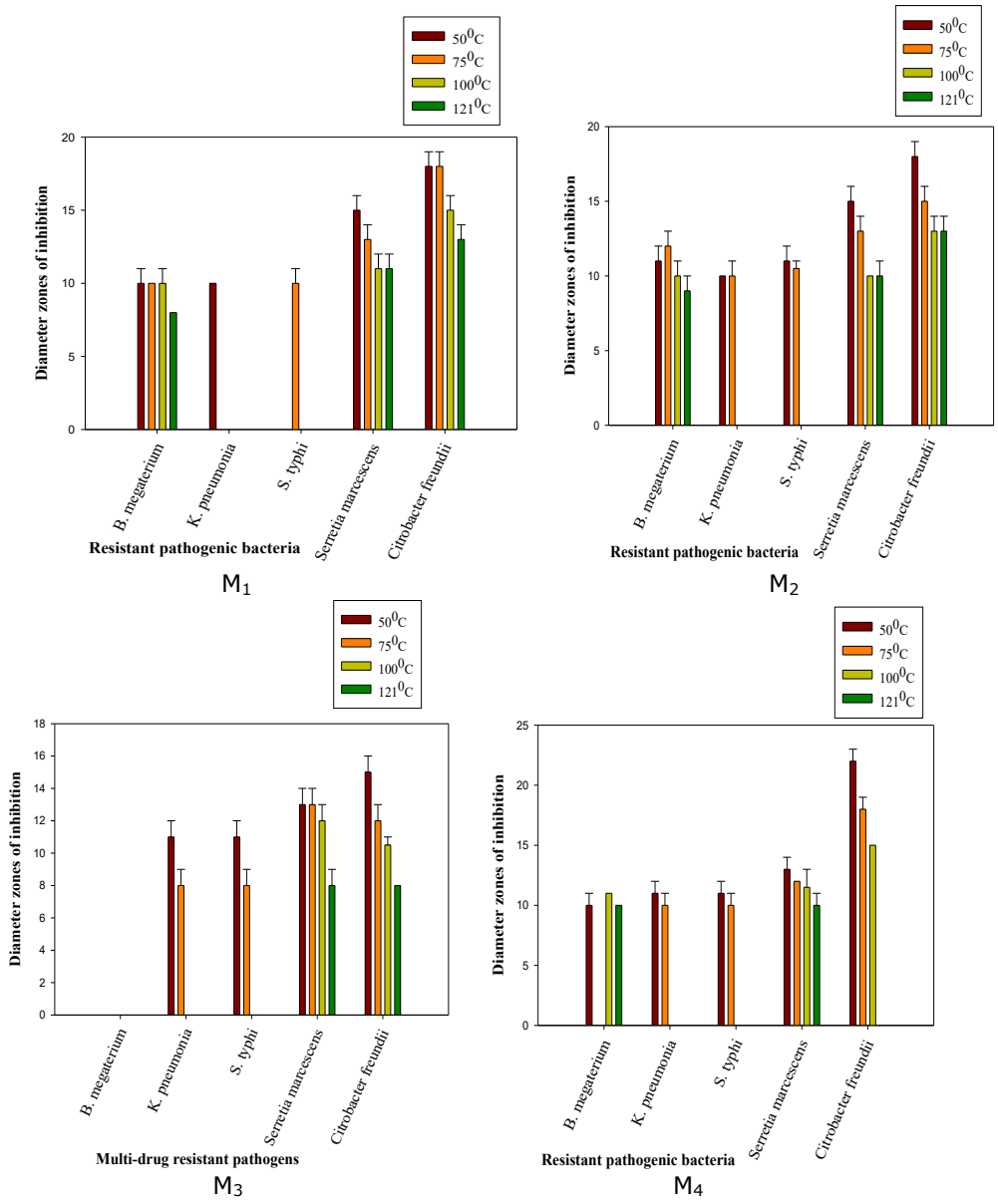


Fig 1: Antibacterial activity of heat-treated partially purified bacteriocins against resistant pathogenic bacteria

Stability testing of bacteriocin-producing LAB:

Effect of heat treatment on bacteriocin*:*

The results showed that bacteriocins produced by the LAB (M_1, M_2, M_3 and M_4) exhibited heat stability at different temperature with the highest at 50^0C, and also exhibited potent antimicrobial activity against *Serratia marcescens*. However, it was observed that there was a reduction in the antimicrobial activity as the temperature increased (Fig 1).

Effect of pH treatment on bacteriocin:

Results show that bacteriocins produced by LAB are more potent at low pH with little or no activity as the pH increased. High antibacterial activity was recorded at pH 2 and pH 4 with no antibacterial activity at pH 9 (Fig 2).

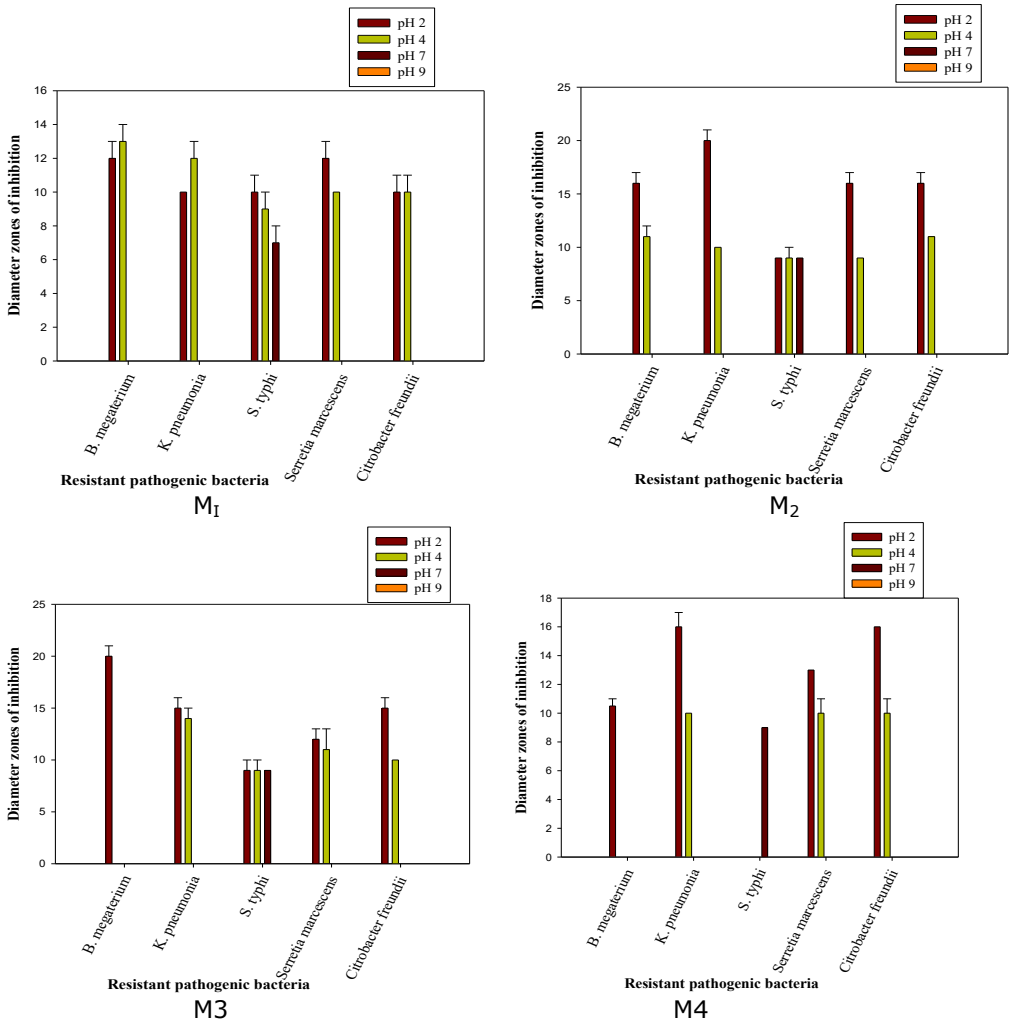


Fig 2: Antibacterial activity of partially purified bacteriocins at different pH against resistant pathogenic bacteria

Sensitivity profile of bacteriocin-producing LAB to proteolytic enzyme (pepsin):

As a result of protein nature of bacteriocin, all purified bacteriocin obtained was fully inactivated (fully sensitive) by proteolytic enzyme, pepsin, hence all the test bacteria were not inhibited (Table 2).

Preservative effect of bacteriocin produced by LAB on cheese and meat:

There was a fairly reduction in microbial load of the food samples subjected to the treatment A (bacteriocin) when compared to other treatment regimen. Treatment B (bacteriocin and refrigeration) showed the lowest microbial load. There was an increase in microbial load in the food products subjected to the treatment of refrigeration only (Treatment C). The food samples in group D (no treatment) had the highest in microbial load as presented in Table 3

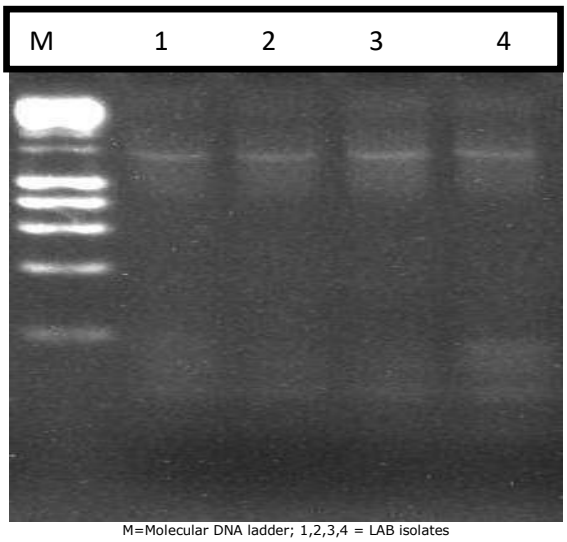
Molecular identification of bacteriocin-producing LAB:

Plate 1 shows the amplified 16s rRNA and DNA molecular ladder, and Fig 1 shows the phylogenetic tree of the isolates. The results from the molecular characterization of the bacteriocin-producing LAB (M_1, M_2, M_3 and M_4) are shown in Table 4.

The four isolates were identified as *Lactobacillus fermentum* strain TSGB1241 for M_1, *Lactobacillus fermentum* strainDMF18 for M_2, *Lactococcus lactis* subsp. *cremoris* C4 DNA for M_3 and *Pediococcus pentosaceus* SL4 for M_4.

Table 2: Sensitivity of bacteriocin-producing LAB to pepsin

Test isolate	**Treated isolated lactic acid bacteria**			
	M1	**M2**	**M3**	**M4**
Salmonella Typhi	Sensitive	Sensitive	Sensitive	Sensitive
Citrobacter freundii	Sensitive	Sensitive	Sensitive	Sensitive
Methicillin resistant *Staphylococcus aureus*	Sensitive	Sensitive	Sensitive	Sensitive
Escherichia coli ATCC 25922	Sensitive	Sensitive	Sensitive	
Bacillus megaterium	Sensitive	Sensitive	Sensitive	Sensitive
Enterococcus faecalis	Sensitive	Sensitive	Sensitive	Sensitive
Serratia marcescens	Sensitive	Sensitive	Sensitive	Sensitive
Klebsiella pneumoniae	Sensitive	Sensitive	Sensitive	Sensitive
MSSA ATCC 25923	Sensitive	Sensitive	Sensitive	Sensitive



M=Molecular DNA ladder; 1,2,3,4 = LAB isolates

Plate 1: Gel electrophoresis picture of amplified 16 sRNA of isolated lactic acid bacteria strains with DNA ladder

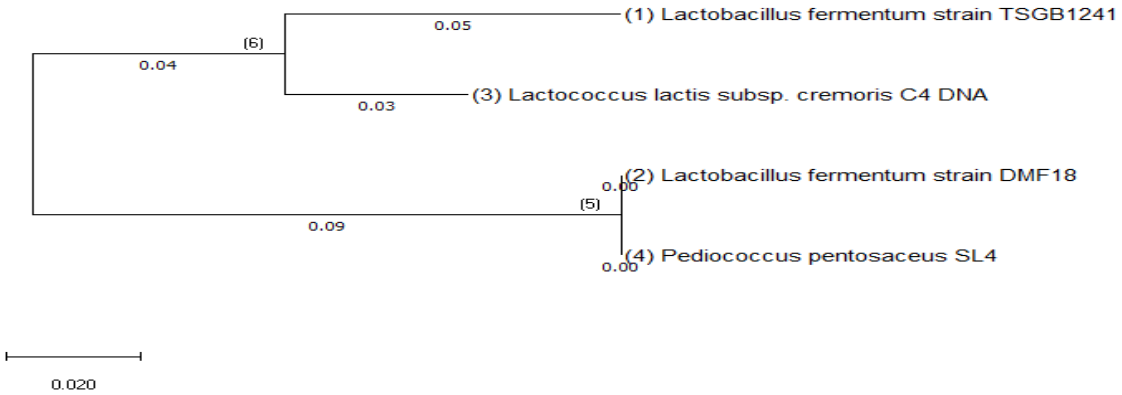


Fig 1: Phylogenetic relationship of identified lactic acid bacteria with those on NCBI

Table 3: Evaluation of microbial loads of food products from preservative effect of LAB bacteriocin on cheese and meat samples

Exposure time (hours)	Mean (±SD) microbial load (x10^7 CFU/ml) in meat after treatment				Mean (±SD) microbial load (x10^7 CFU/ml) in cheese after treatment			
	A	B	C	D	A	B	C	D
24	15±1.41^a	40±0.00^b	135.5±2.12^c	140.5±2.12	32±0.71^b	15±1.41^{bc}	50±7.07^b	30±0.00
48	30±2.82^a	16±2.83^a	120.5±2.12^c	4±1.41	40±7.07^c	3.5±2.12^a	30±14.14^{ab}	25±4.24
72	20±7.07^a	50±2.83^c	80.5±7.78^b	250.5±28.99	25±2.83^b	20.5±3.54^c	8±5.66^a	80±8.49
96	31±0.00^a	43±1.41^b	50±0.00^a	125.5±0.71	20±0.00^{ab}	12.5±0.71^{abc}	11±1.41^a	115±0.00
110	30±7.07^a	17±1.41^a	84.5±13.44^b	TNTC	10±4.24^a	8±4.24^{ab}	16.5±0.71^a	TNTC

A = Treatment with bacteriocin only; B = Bacteriocin and refrigeration; C = Refrigeration only; D = Without bacteriocin and refrigeration; TNTC = Too numerous to count
Note: Alphabets in the superscript are statistically different at 0.05 probability level

Table 4: Molecular identification of lactic acid bacteria (M_1, M_2, M_3 and M_4)

Organism name	Maximum score	E-value	Identity (%)	Accession number
Lactobacillus fermentum strain TSGB1241	1646	0.0	95.75	MN255729.1
Lactobacillus fermentum strain DMF18	1105	0.0	92.08	MH782089.1
Lactococcus lactis subsp. cremoris C4 DNA	728	0.0	81.38	AP018499.1
Pediococcus pentosaceus SL4	710	0.0	83.82	CP006854.1

Discussion:

The use of antimicrobial peptide produced by lactic acid bacteria for bio-preservation is a relatively new concept to eliminate or control the spread of pathogenic or spoilage bacteria in food (16). This study has successfully isolated bacteriocin-producing LAB from lactic acid bacteria isolated from dairy products with potential for bio-preservation. *Lactobacillus* and *Streptococcus* represent the genera of bacteria capable of producing bacteriocin. The morphological and biochemical features of the LAB demonstrated in this study are in line with the previous study by Soltan et al., (9) who also identified these genera of bacteria capable of producing bacteriocin.

Heat stability suggested that bacteriocins produced by LAB in this study are able to tolerate different heat treatment (temperature). This is similar to the observation made by Wu et al., (17) who purified and characterize bacteriocin produced by a strain of *Lacticaseibacillus rhamnosus* ZFM216 and also observed stability of the bacteriocin at different temperature (17). However, the observation that antibacterial activity reduced with increase in temperature may be attributed to the fact that high temperature partially affects some components of bacteriocin. Bacteriocin are proteinaceous in nature and protein are denatured by high temperature. The antimicrobial activity of the bacterial isolates studied were observed to have maximum activity at acidic pH (2.0–4.0) and a reduction in antibacterial activity at pH 7 and no activity at pH 9. This result suggests that such bacteriocins could be used in acidic food products like fruit juices and food products that has neutral pH. This result is in consonance with the findings of Singh et al*.,* (8), whose study showed maximum bactericocin activity at pH 2-6. High antimicrobial activity was detected at low pH in this study, as also shown by Araújo et al*.,* (19), but contrast the study of Abanoz and Kundholu (20), who reported in a study that bacteriocin activity are maintained over a wide range of temperature.

Also, the bacteriocin treated with proteolytic enzyme (pepsin) lost antibacterial potential, implying that proteolytic enzyme was able to degrade the bacteriocin. Soltani et al., (21) reported similarly that bacteriocin from Gram-positive and Gram-negative bacteria have tendency of degrading bacteriocin (21). This indicates that bacteriocins are proteins. The food products (cheese and meat) treated with bacteriocin had a reduced microbial load when compared to the food products that were refrigerated alone. This is because food spoilage organism such as mesophilic bacteria can be inhibited by refrigeration.

The molecular analysis of the LAB isolated from the food products revealed M1 to be *Lactobacillus fermentum* strain TSGB 1241, M2 to be *Lactococcus fermentum* strain DMF 18, M2 to be *Lactococcus lactis* subsp. *cremoris* and M4 to be *Pediococcus pentosaceus* SL4. The four LAB strains isolated were identified using 16S rRNA sequencing followed by BLAST analyzing. Sanger sequencing by 16S rRNA is a good option due to the possession of proper gene size and availability of a large number of sequences in databases for comparison. The phylogenetic tree of the characterized bacteriocin producing LAB constructed revealed the closest identity to those in the GenBank through neighbour-joining method (22).

Conclusion:

This study identified bacteriocins from four LAB which exhibited stability and preservative potential against resistant bacterial pathogens in meat and cheese. it is recommended that bacteriocin-producing LAB be considered as alternative to combat resistant pathogenic bacteria in addition being used as natural, safe and effective bio-preservatives.

Contribution of authors:

SAO conceptualized and planned the methodology; OIO wrote the manuscript and was involved in conduct of the study; OOG analyzed and presented the result of the study; and STA awas involved in conducting laboratory study and data collection. All the authors contributed to manuscript and approved the manuscript submitted for publication.

Conflict of interest:

Authors declare that no known conflict of interest or personal relationships that could have influence the work reported in this paper.

Data availability:

Data are available upon request from the corresponding author of this paper.

Source of funding:

Authors of this study received no funding from any government or private organization.

References:

1. Duarte-Mata, D. I., and Salinas-Carmona, M. C. Antimicrobial peptides´ immune modulation role in intracellular bacterial infection. Front Immunol. 2023; 14: 1119574. doi: 10.3389/fimmu.2023.1119574.
2. Reddy, K. V. P., Yedery, R. D., and Aranha, C. Antimicrobial peptides: An emerging category of therapeutic agents. Curr Opin Biotech. 2004; 15 (5): 316–322. https://doi.org/10.1016/j.ijantimicag.2004.09.005
3. Li, H., Niu, J., Wang, X., et al. The Contribution of Antimicrobial Peptides to Immune Cell Function: A Review of Recent Advances. Pharmaceutics. 2023; 15(9):2278.doi: 10.3390/pharmaceutics15092278
4. Wang, G., Mishra, B., Lau, K., et al. Antimicrobial peptides in 2014. Pharmaceuticals. 2015; 8 (1): 123-150. doi: 10.3390/ph8010123.
5. Mishra, B., Reiling, S., Zarena, D., et al. Host defense antimicrobial peptides as antibiotics: Design and application strategies. Curr Opin Chem Biol. 2017; 38: 87–96. doi: 10.1016/j.cbpa.2017.03.014
6. Wang, Z., and Wang, G. APD3: The Antimicrobial Peptide Database as a Tool for Research and Education. Nucl Acid Res. 2016; 44: D1087-D1093. https://doi.org/10.1093/nar/gkv1278
7. Heruka De Zoysa, G., Cameron, A. J., Hegde, V. V., et al. Antimicrobial Peptides with Potential for Biofilm Eradication: Synthesis and Structure Activity Relationship Studies of Battacin Peptides. J Med Chem. 2015; 58: 625–639. doi: 10.1021/jm501084q
8. Gupta, R., Jeevaratnam, K., and Fatima, A. Lactic Acid Bacteria: Probiotic Characteristic, Selection Criteria, and its Role in Human Health—A Review. Int J Environ Res Publ Health. 2018; 5 (10): 411-424
9. Soltan-Dallal, M. M., Zamaniahari, S., Davoodabadi, A., et al. Identification and characterization of probiotic lactic acid bacteria isolated from traditional Persian pickled vegetables. GMS Hyg Infect Contr. 2017; 12: 15. doi: 10.3205/dgkh000300.
10. Hammes, W. P., and Hertel, C. The Genera Lactobacillus and Carnobacterium. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., and Stackebrandt, E (Eds.). The Prokaryotes. 3rd edn., Vol. 4: Bacteria: Firmicutes, Cyanobacteria, Springer, New York. 2006: 320-403. doi: 10.1007/0-387-30744-3_10
11. Sajid, I., Muhammad, B., Farida, R., et al. Screening, Characterization and Optimization of antibacterial peptides, produced by *Bacillus safensis* strain MK-12 isolated from waste dump soil KP. Pakistan. 2018; doi.org/10.1101/308205.
12. Bergey, D. H., and Holt, J. G. Bergey's Manual of Determinative Bacteriology. 9th edn. Philadelphia: Lippincott Williams & Wilkins. 2000.
13. Gautam, N., and Sharma, N. Bacteriocin: safest approach to preserve food products. Indian J Microbiol. 2009; 49(3): 204–211. doi: 10.1007/s12088-009-0048-3.
14. Fugaban, J. I. I., Vazquez Bucheli, J. E., Holzapfel, W. H., et al. Characterization of Partially Purified Bacteriocins Produced by *Enterococcus faecium* Strains Isolated from Soybean Paste Active Against *Listeria* spp. and Vancomycin-Resistant Enterococci. Microorganisms. 2021;9 (5):1085. doi: 10.3390/microorganisms9051085
15. Kumar, S., Stecher, G., Li, M., et al. MEGAX: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018; 35 (6): 1547-1549. doi: 10.1093/molbev/msy096.
16. Putri, D. A., Lei, J., Rossiana, N., et al. Bio-preservation of Food Using Bacteriocins From Lactic Acid Bacteria: Classification, Mechanisms and Commercial Applications. Int J Microb. 2024: 8723968. doi: 10.1155/ijm/8723968.
17. Wu, D., Dai, M., Shi, Y., et al. Purification and characterization of bacteriocin produced by a strain of *Lacticaseibacillus rhamnosus* ZFM216. Front Microbiol. 2022; 13: 1050807. doi: 10.3389/fmicb.2022.1050807
18. Singh, J. K., Devi, P. B., Reddy, G. B., et al. Biosynthesis, classification, properties, and applications of *Weissella* bacteriocins. Front Microbiol. 2024; 15: 1406904. doi: 10.3389/fmicb.2024.1406904.
19. Araújo, E. S., Pimenta, A. S., Feijó, F. M. C., et al. Antibacterial and antifungal activities of pyroligneous acid from wood of *Eucalyptus urograndis* and *Mimosa tenuiflora*. J Appl Microb. 2018; 124 (1): 85-96. doi: 10.1111/jam.13626
20. Abanoz, H. S., and Kunduhoglu, B. Antimicrobial activity of Bacteriocin produced by *Enterococcus faecalis* KT11 against some pathogens and Antibiotic-resistant bacteria. Korean J Food Animal. 2018; 38 (5): 1064-1079 doi: 10.5851/kosfa.2018.e40.
21. Soltani, S., Zirah, S., Rebuffat, S., et al. Gastrointestinal Stability and Cytotoxicity of Bacteriocins from Gram-Positive and Gram-Negative Bacteria: A Comparative *in vitro* study. Front Microbiol. 2022; 12: 780355. doi: 10.3389/fmicb.2021.780355
22. Elayaraja, S., Annamalai, N., Mayavu, P., et al. Production, purification and characterization of bacteriocin from Lactobacillus murinus AU06 and its broad antibacterial spectrum. Asian Pac Trop Biomed. 2014; 4 (Suppl 1): S305–S311. https://doi.org/10.12980/APJTB.4.2014C537

Onyekwere et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 361 - 370 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2495. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Antibiofilm activity of plant lectins: Evaluating the anti-adhesion properties of *Treculia africana*, *Moringa oleifera*, and *Allium sativum* on clinical bacterial isolates

[1]Onyekwere, U. U., [*2]Eluu, S. C., [3]Eluu, K., [4]Aina, T., [5]Enyigwe, J. O., [6]Okoye, C. S., [2]Nwankwo, U. V., [7]Burba, R. A., [4]Akinbiola, A. B., [8]Oke, B., [9]Ebem, E. C., and [10]Eze, G. O.

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, 420110, Agulu, Anambra State, Nigeria
[2]Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
[3]Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria
[4]Department of Biomedical Engineering, Afe Babalola University Ado-Ekiti, KM 8.5 Afe Babalola Way, Ado-Ekiti, Nigeria
[5]Department of Agricultural Economics, Management and Extension, Ebonyi State University, Abakaliki, Nigeria
[6]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences. David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
[7]Department of Family and Consumer Science, Faculty of Agriculture, Taraba State University Jalingo, Nigeria
[8]Applied Microbiology Department, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
[9]Department of Biotechnology, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Abakaliki, Ebonyi State, Nigeria
[10]Department of Scientific and Research Services, David Umahi Federal University Teaching Hospital, Uburu, Ebonyi State, Nigeria
*Correspondence to: eluustanleychijioke@gmail.com

Abstract:

Background: The increasing prevalence of biofilm-associated infections caused by multidrug-resistant organisms has intensified the search for alternative antimicrobial agents. This study investigated the antibiofilm activity of lectins isolated from *Treculia africana* leaves (TAL), *Moringa oleifera* leaves (MOL), and *Allium sativum* bulbs (ASL) against selected clinical bacterial isolates.
Methodology: Lectins were extracted from the three plant parts using modified conventional crude extraction methods. The antibiofilm activities against clinical *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* isolates were evaluated at extract concentrations of 500µg/ml, 100µg/ml and 10µg/ml in a 96-well U-bottom microtitre plates for 4 hours and 24 hours, by biofilm inhibition and quantification assays. The antibiofilm activity, performed in triplicates, was expressed as percentage inhibition, and classified as weak (<40%), moderate (40–60%), or strong (>60%).
Results: The result showed that 500µg/ml TAL lectin extract at 4-hour incubation showed moderate anti-biofilm activity against *S. aureus* (41% inhibition), but minimal or no activity against other isolates. At 24-hour incubation, TAL extract produced moderate anti-biofilm activity against *K. pneumoniae* (43%) and *B. subtilis* (43.3%), with weak activity against *S. aureus* (19%) and *P. aeruginosa* (17.3%). MOL lectin extract produced stronger activity than TAL extract, reducing biofilm adhesion at 500µg/ml by 35–66% across all isolates after 4 hours and up to 57% after 24 hours, particularly against *K. pneumoniae* and *B. subtilis*. ASL extract produced selective antibi-biofilm activity, mainly against *S. aureus* (37% at 4 h; 22.3% at 24 h) and *P. aeruginosa* (42.3% at 24 h), with little or no effect on other isolates. Across all lectins, antibiofilm activity decreased with lower concentrations, and none completely inhibited bacterial adhesion.
Conclusion: The result indicates the promise of these plant-based lectins as a natural agent for controlling biofilm-associated infections.

Keywords: Plant lectins; Antibiofilm; Multidrug-resistant; *Moringa oleifera*; *Treculia africana*; *Allium sativum*

Received Feb 12, 2026; Revised Apr 26, 2026; Accepted Apr 27, 2026

Activité antibiofilm des lectines végétales: Évaluation des propriétés anti-adhésives de *Treculia africana, Moringa oleifera* et *Allium*

sativum sur des isolats bactériens cliniques

[1]Onyekwere, U. U., *[2]Eluu, S. C., [3]Eluu, K., [4]Aina, T., [5]Enyigwe, J. O., [6]Okoye, C. S., [2]Nwankwo, U. V., [7]Burba, R. A., [4]Akinbiola, A. B., [8]Oke, B., [9]Ebem, E. C., et [10]Eze, G. O.

[1]Département de Microbiologie et de Biotechnologie Pharmaceutiques, Faculté des Sciences Pharmaceutiques, Université Nnamdi Azikiwe, 420110, Agulu, État d'Anambra, Nigéria
[2]Département de Biotechnologie, Faculté des Sciences, Université d'État d'Ebonyi, Abakaliki, Nigéria
[3]Département de Génétique et Biotechnologie, Faculté des Sciences Biologiques, Université de Calabar, Calabar, Nigéria
[4]Département de Ingénierie Biomédecine, Université Afe Babalola Ado-Ekiti, Km 8.5 Afe Babalola Way, Ado-Ekiti, Nigéria
[5]Département d'Economie, de Gestion et de Vulgarisation Agricoles, Université d'État d'Ebonyi, Abakaliki, Nigéria
[6]Département de Pharmacie et de Pharmacie Industrielle, Faculté des Sciences Pharmaceutiques, Université Fédérale des Sciences de la Santé David Umahi, Uburu, État d'Ebonyi, Nigéria
[7]Département des Sciences de la Famille et de la Consommation, Faculté d'Agriculture, Université d'État de Taraba, Jalingo, Nigéria
[8]Département de Microbiologie Appliquée, Faculté des Sciences, Université d'État d'Ebonyi, Abakaliki, Nigéria
[9]Département de Biotechnologie, Université Fédérale Alex Ekwueme, Ndufu-Alike Ikwo, Abakaliki, État d'Ebonyi, Nigéria
[10]Département des Services Scientifiques et de Recherche, Hôpital Universitaire Fédéral David Umahi, Uburu, État d'Ebonyi, Nigéria
*Correspondance à: eluustanleychijioke@gmail.com

Résumé:

Contexte: La prévalence croissante des infections associées aux biofilms causées par des organismes multirésistants a intensifié la recherche d'agents antimicrobiens alternatifs. Cette étude a examiné l'activité antibiofilm de lectines isolées de feuilles de *Treculia africana* (TAL), de feuilles de *Moringa oleifera* (MOL) et de bulbes d'*Allium sativum* (ASL) contre des souches bactériennes cliniques sélectionnées.
Méthodologie: Les lectines ont été extraites des trois parties de la plante par une méthode d'extraction brute conventionnelle modifiée. L'activité antibiofilm contre des souches cliniques de *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* et *Bacillus subtilis* a été évaluée à des concentrations d'extrait de 500µg/ml, 100µg/ml et 10µg/ml dans des plaques de microtitration à 96 puits à fond en U, pendant 4 heures et 24 heures, par des tests d'inhibition et de quantification du biofilm. L'activité antibiofilm, réalisée en triplicata, a été exprimée en pourcentage d'inhibition et classée comme faible (<40%), modérée (40–60%) ou forte (> 60%).
Résultats: Après 4 heures d'incubation, l'extrait de lectine TAL (500µg/ml) a présenté une activité anti-biofilm modérée contre *S. aureus* (41% d'inhibition), mais une activité minimale, voire nulle, contre les autres souches. Après 24 heures d'incubation, l'extrait TAL a induit une activité anti-biofilm modérée contre *K. pneumoniae* (43%) et *B. subtilis* (43,3%), et une faible activité contre *S. aureus* (19%) et *P. aeruginosa* (17,3%). L'extrait de lectine MOL a présenté une activité supérieure à celle de l'extrait TAL, réduisant l'adhérence du biofilm de 35 à 66% pour toutes les souches après 4 heures et jusqu'à 57% après 24 heures à la concentration de 500µg/ml, notamment contre *K. pneumoniae* et *B. subtilis*. L'extrait d'ASL a présenté une activité antibiofilm sélective, principalement contre *S. aureus* (37% à 4 h; 22,3% à 24 h) et *P. aeruginosa* (42,3% à 24 h), avec peu ou pas d'effet sur les autres isolats. Pour toutes les lectines testées, l'activité antibiofilm a diminué avec la concentration, et aucune n'a inhibé complètement l'adhésion bactérienne.
Conclusion: Ces résultats suggèrent le potentiel de ces lectines d'origine végétale comme agent naturel pour lutter contre les infections associées aux biofilms.

Mots-clés: Lectines végétales; Antibiofilm; Multirésistance; *Moringa oleifera*; *Treculia africana*; *Allium sativum*

Introduction:

Biofilms are common in nature because they represent a survival strategy used by bacteria (1). Microbial biofilms represent a major challenge in both clinical and industrial settings due to their exceptional resistance to antimicrobial agents and disinfectants (2,3). Biofilms are structured communities of microbial cells enclosed in a self-produced extracellular polymeric matrix that adheres to biotic or abiotic surfaces (4). Clusters of bacteria embedded in a self-produced matrix and affixed to a surface or one another are known as bacterial biofilms (1). This sessile mode of growth provides microorganisms with enhanced tolerance to hostile environmental conditions, antibiotics, and host immune responses, contributing to persistent infections and increased morbidity and mortality rates (5). In clinical environments, biofilm-associated infections are implicated in up to 80% of chronic microbial diseases, including those involving indwelling medical devices such as catheters, prostheses, and implants (6).

The expression levels of many genes linked to the maturation and generation of exopolysaccharide (EPS), commonly referred to as "slime" or bacterial EPS, rapidly alter when bacteria adhere to the surface (7). The existence of a self-produced EPS and limited motility make

the adhesion stable, irreversible, and permanent if cells stay on the substrate surface for a long time without any physical or chemical interference (8,9). Therefore, preventing bacterial attachment has emerged as a promising strategy for controlling biofilm development, offering an advantage over conventional antimicrobial therapies that primarily target planktonic cells. This has spurred interest in identifying natural anti-adhesion agents capable of disrupting bacterial colonization without exerting selective pressure for resistance.

Compounds and extracts derived from this plant exhibit a wide range of biological activities, including oxytocic effects, modulation of the central nervous system, anti-inflammatory action, anti-tubercular properties, anti-ulcer activity, hepato-renal protective effects, as well as anti-trypanosomal, anticancer, and antidiabetic potentials (10,11). Plant-derived lectins, a diverse group of carbohydrate-binding proteins, have attracted attention as potential antibiofilm and anti-adhesion agents due to their ability to recognize and bind specific sugar moieties on microbial cell surfaces (12). These interactions can block key receptors involved in adhesion, interfere with biofilm matrix synthesis, and inhibit cell-to-cell communication (quorum sensing) (13,14).

Several studies have demonstrated that lectins from various plant sources exhibit inhibitory effects on pathogenic bacteria by preventing their attachment to epithelial cells or abiotic surfaces (15,16). Widely prevalent throughout West and Central Africa, *Treculia africana* (African breadfruit) yields 20–30 pods of edible seeds annually and is a delicacy but underutilized food security crop among the Igbo people of southeast Nigeria (17). *Moringa oleifera* (drumstick or horseradish tree) is native to India but grows well in tropical and subtropical climates because it can withstand drought and moderate winter. All parts of the plant have nutritional and commercial value (18). *Allium sativum* is a highly prized culinary and therapeutic herb with a variety of pharmacological qualities, such as anti-inflammatory, anti-cancer, antioxidant, and antibacterial activities. This study investigates the anti-adhesion activities of lectins extracted from *T. africana*, *M. oleifera*, and *A. sativum* against selected bacterial pathogens.

Materials and method:

Culture media and antibiotics:

Culture media used were Trypton Soy broth (Oxoid, UK), Mueller-Hinton (MH) agar (Oxoid, UK), nutrient agar, and Trypton soy agar powder (Oxoid, UK). All culture media were prepared aseptically in line with the manufacturers' instructions. The antibiotic powder used in this work was ciprofloxacin which was a gift from Juhel Nigeria ltd.

Microorganisms:

The bacterial strains used in this study comprised two Gram-positive species (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative species (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). These clinical isolates were sourced from the laboratory of the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria. Each isolate was initially grown in Tryptone Soy broth and subsequently sub-cultured onto Tryptone Soy agar chosen for its suitability in supporting biofilm formation. Stock cultures were maintained on Tryptone Soy agar slants and refrigerated at 4–6 °C.

Plant materials:

Fresh, mature *Moringa oleifera* leaves were sourced from the Abakpa Nike community in Enugu State, and *Allium sativum* (garlic) bulbs together with *Treculia africana* (African breadfruit) seeds were purchased at Ogbete Main Market, Enugu State, Nigeria. All plant specimens were taxonomically identified by Dr. C. S. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.

Extraction of lectins from plant materials:

Using modified conventional methods, crude lectin proteins were isolated from *T. africana*, *A. sativum*, and *M. oleifera*. With modifications, the extraction procedure for *M. oleifera* was carried out in accordance with Khatun et al., (19). A 0.2 M NaCl solution (pH 6.5) containing polyvinylpyrrolidone (PVP) was combined with dried leaf powder, left overnight at 4°C, centrifuged, and the supernatant precipitated with 100% saturated ammonium sulfate. After dialyzing the precipitate against Tris-HCl buffer (pH 8.4) and distilled water, it was lyophilized.

The extraction process for *T. africana* was carried out using the modified approach proposed by Laija et al., (20). Seed flour that had been defatted was extracted at 4°C in phosphate-buffered saline (PBS, pH 7.2). Proteins were dialyzed, lyophilized, and separated using ammonium sulfate precipitation (40% and 60% saturation). Ground bulbs of *A. sativum* were mixed with NaCl in PBS (pH 7.4), centrifuged, and the resulting supernatant precipitated with 70% ammonium sulfate. The protein pellet was dialyzed and lyophilized.

Preparation of stock solution and standardization of test bacteria:

For antibiofilm and anti-adhesion assay, stock solution of the lectin extracts were prepared by dissolving 1000mg of each extract in 5ml of sterile Tryptone Soy broth to obtain a concentration of 200 mg/ml. A 0.5 McFarland turbidity standard, equivalent to 1.5×10^8 CFU/ml, was prepared from barium chloride and sulfuric acid solutions and used to standardize bacterial inocula. Test bacteria were adjusted to match the 0.5 McFarland standard by suspending overnight cultures in sterile saline to achieve a final population of approximately 10^8 CFU/ml before use in antimicrobial assays.

Determination of antibiofilm activity of the plant lectin extracts:

For the determination of anti-adhesion properties, different concentrations of the three plant lectin extracts were prepared from the stock solution at 50mg/ml, 10mg/ml, and 1mg/ml. Following the addition of 100µl of each extract to the 96-well U-bottom microtitre plates, each well received an equivalent volume of 100 µl of standardized culture (1.0×10^6 CFU/ml) of every test bacterium, resulting in a final volume of 200µl and final concentrations of 500µg/ml, 100µg/ml and 10µg/ml. Approximately 2.5µg/ml of ciprofloxacin served as the positive control, while 2.5µg/ml of TSB and the test bacteria served as the negative control. Three duplicates of each procedure were performed.

To promote cell attachment and biofilm formation, the plates were sealed with sterile sealant and incubated at 37^{o}C for 4 and 24 hrs, respectively, in a humidified container without shaking. The quantification of biofilm biomass was evaluated using the crystal violet staining method after incubation.

Assessment of biofilm biomass using crystal violet quantification assay:

Using modified crystal violet (CV) staining test as described by Stepanovic et al., (21), cell attachment was indirectly evaluated. To get rid of weakly attached cells, the plates were rinsed three times with sterile distilled water after incubation. After 30 minutes of air drying, 200µl of methanol was applied to each well to fix the remaining attached cells for 15 minutes.

The staining was done for five minutes using 200µl of 1% crystal violet after the plates had been emptied and allowed to air dry. Plates were oven-dried at 60^{O}C for 45 minutes after excess stains were removed with a running tap. Once the cells had dried, 200µl of 33% glacial acetic acid was used to resolubilize the dyes that had stuck to them.

The optical density of each destained well was then measured at 640 nm using an ELISA microtitre plate reader after 100µl of each well had been transferred to a fresh plate. The percentage inhibition was classified as weak (<40%), moderate (40–60%), or strong (>60 %) reduction, and calculated using the formula; % inhibition = (OD of experimental / OD of negative control) × 100, where OD is optical density, and % reduction = 100 – % inhibition.

Statistical analysis:

Data on antibiofilm activity were presented as percentage inhibition, and graphs were generated using Origin software.

Results

Antiadhesion property of *Treculia africana* leaf (TAL) lectins at 4 and 24 hours:

The result of the anti-adhesion activity of TAL lectin after 4 hours exposure is shown in Fig 1a. Complete inhibition of biofilm formation was not achieved by any treatment, including the positive control (ciprofloxacin, 2.5µg/ml), with some samples showing no detectable inhibition activity. At 500µg/ml, TAL produced percentage reductions in biofilm adhesion of *K. pneumoniae* (0%), *B. subtilis* (11%), *S. aureus* (41%), and *P. aeruginosa* (19%). At 100µg/ml, inhibition was absent (0%) in *K. pneumoniae* and *P. aeruginosa*, while *B. subtilis* and *S. aureus* showed reductions of 6% and 28%, respectively. At 10µg/ml, no inhibition (0%) was observed in any of the isolates except *S. aureus*, which exhibited 20% reduction. The positive control demonstrated weak anti-adhesion activity (<40% reduction).

The result of anti-adhesion activity of TAL after 24 hours exposure is shown in Fig 1b. None of the tested lectins completely inhibited bacterial attachment, including the positive control (ciprofloxacin), and some samples exhibited no detectable anti-adhesion effect. At concentration of 500µg/ml, TAL produced percentage reductions in biofilm adhesion as follows; *K. pneumoniae* (43%), *B. subtilis* (43.3%), *S. aureus* (19%), and *P. aeruginosa* (17.3%). When the concentration was reduced to 100µg/ml, inhibition levels were 8.3% for *K. pneumoniae*, 25% for *B. subtilis*, and 21% for *P. aeruginosa*, while *S. aureus* showed no measurable activity. At the lowest concentration (10µg/ml), reductions were observed in *K. pneumoniae* (14%) and *P. aeruginosa* (12%), with no anti-adhesion effect detected against *B. subtilis* or *S. aureus*.

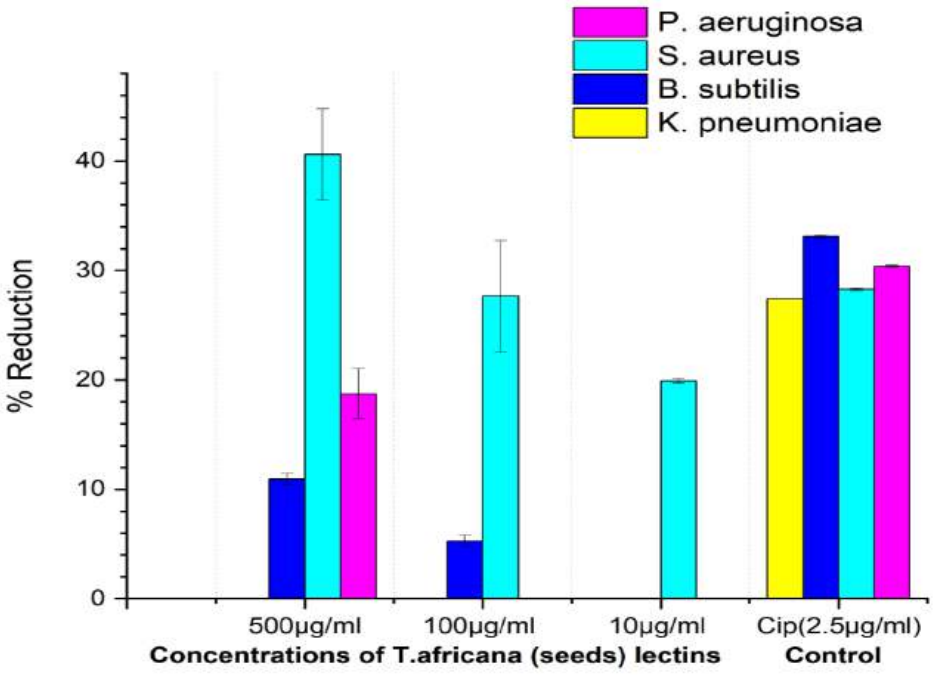


Fig 1a: Anti-adhesion property of *Treculia africana* (seeds) lectins at 4 hours exposure

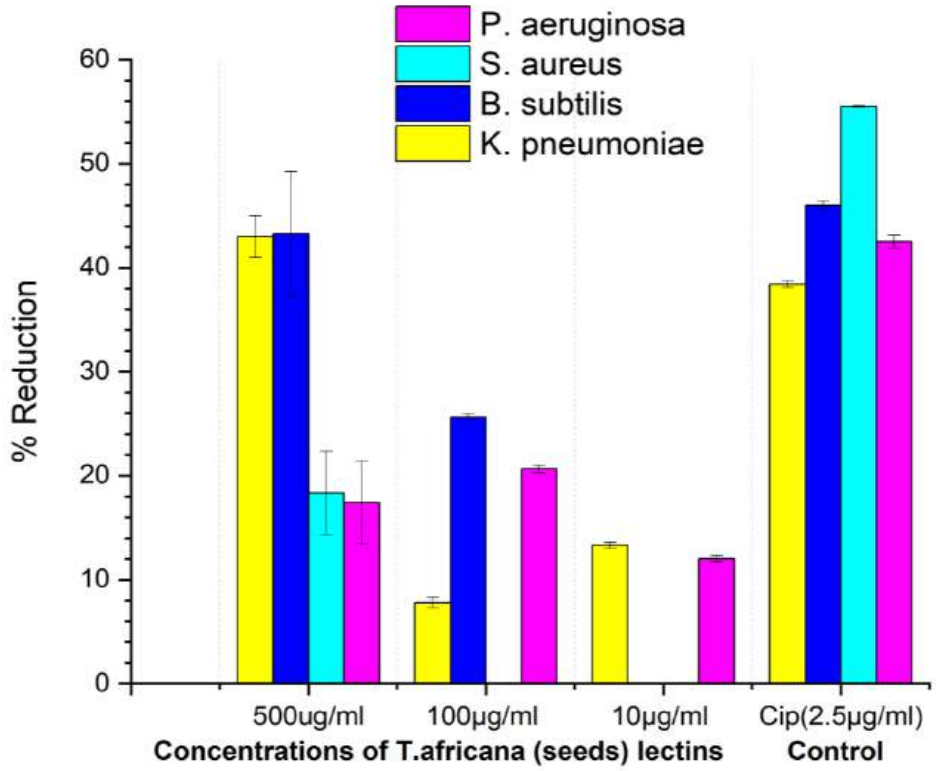


Fig 1b: Antiadhesion property of *Treculia africana* (seeds) lectins at 24 hours exposure

Anti-adhesion property of *Moringa oleifera* leaf (TAL) lectins at 4 and 24 hours:

The anti-adhesion potential of MOL lectin after 4 hours of exposure is shown in Fig 2a. Complete inhibition of biofilm attachment was not achieved by any of the treatments, including the positive control (ciprofloxacin, 2.5µg/ml), while certain samples exhibited no detectable anti-adhesion activity. At a concentration of 500µg/ml, MOL lectin reduced bacterial adhesion by 35% for *K. pneumoniae*, 36.4% for *B. subtilis*, 27% for *S. aureus*, and 66% for *P. aeruginosa*. When the concentration was reduced to 100µg/ml, *K. pneumoniae* and *S. aureus* showed no inhibition, whereas *B. subtilis* and *P. aeruginosa* exhibited 16% and 40% reductions, respectively. At the lowest concentration (10µg/ml), MOL lectin decreased adhesion by 27% in *K. pneumoniae*, 39% in *B. subtilis*, and 36% in *P. aeruginosa*, but had no observable effect on *S. aureus*.

The anti-adhesion effect of MOL lectin after 24 hours of treatment is as shown in Fig 2b. At a concentration of 500µg/ml, MOL lectin produced percentage reductions in biofilm adhesion as follows; *K. pneumoniae* (57%), *B. subtilis* (50%), *S. aureus* (39%), and *P. aeruginosa* (3%). At 100µg/ml, inhibition levels were *K. pneumoniae* (29%), *B. subtilis* (7.3%), *S. aureus* (19.3%), and *P. aeruginosa* (8.3%). At the lowest concentration of 10µg/ml, the reductions recorded were *K. pneumoniae* (35%), *B. subtilis* (15%), and *S. aureus* (16%), while *P. aeruginosa* showed no detectable anti-adhesion activity. None of the lectin extracts, including the positive control (ciprofloxacin, 2.5µg/ml), was able to completely prevent bacterial attachment, and in some cases, certain lectins appeared to promote cell adhesion rather than inhibit it.

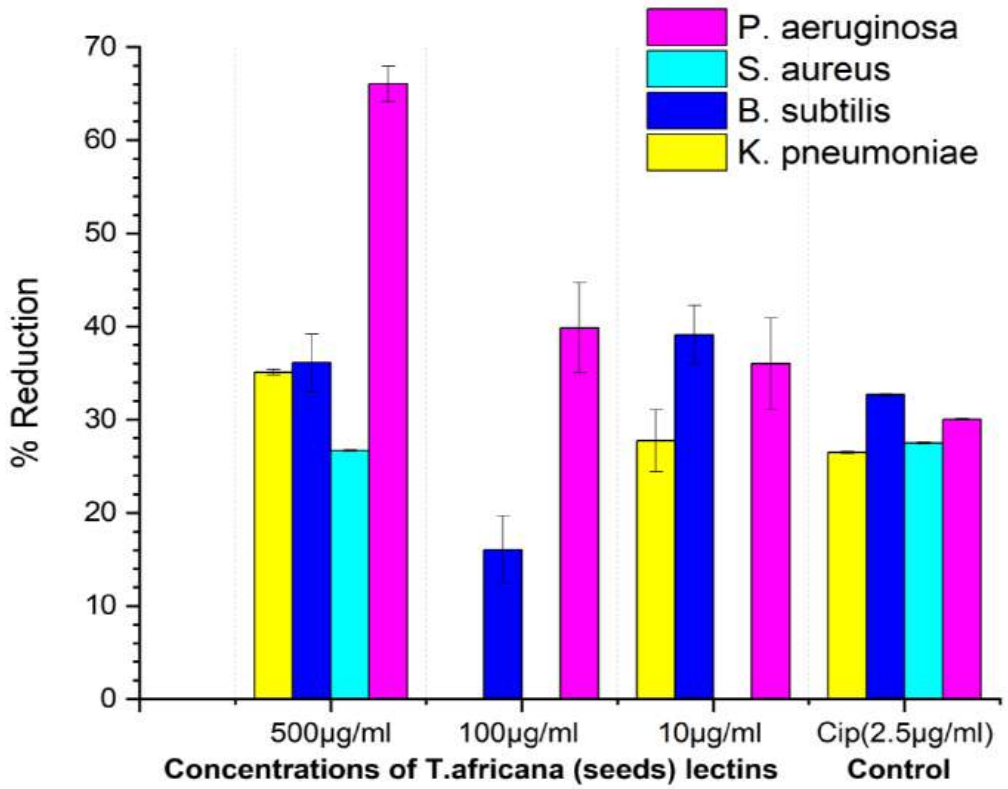


Figure 2a: Antiadhesion property of *Moringa oleifera* (leaves) lectins (in % reduction) at 4 hours exposure

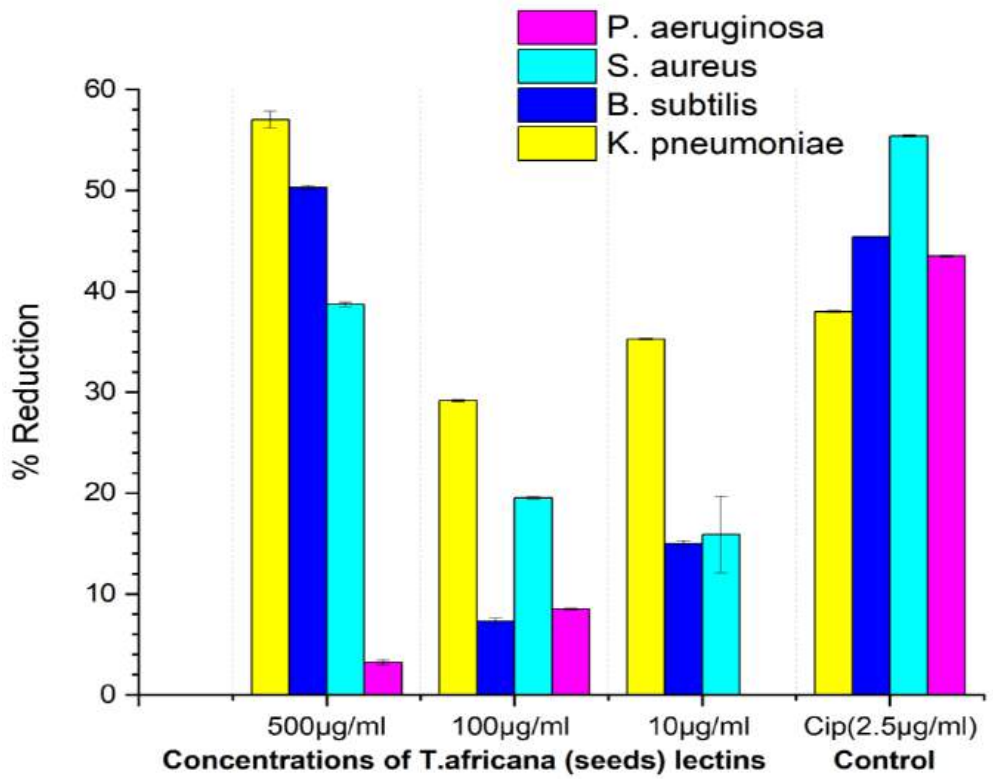


Figure 2b: Antiadhesion property of *Moringa oleifera* (leaves) lectins at 24 hours exposure

Anti-adhesion property of *Allium sativum* bulbs (ASL) lectins at 4 and 24 hours:

The effect of ASL lectin in inhibition of biofilm cell adhesion (at 4 hours) is shown in Fig 3**.** At a concentration of 500µg/ml and after 4 hours of exposure, the following anti-adhesion effects on the tested bacterial isolates were; *K. pneumoniae* (0%), *B. subtilis* (0%), *P. aeruginosa* (0%), and *S. aureus* (37%) (Fig. 3a). When the concentration was reduced to 100µg/ml, no inhibitory activity was observed against any of the isolates except *S. aureus*, which showed a 19% decrease in biofilm adhesion. At 10µg/ml, ASL lectin displayed no detectable anti-adhesion effect on any of the bacterial species. Notably, none of the plant lectins, including the positive control, ciprofloxacin (2.5µg/ml) completely prevented bacterial cell attachment.

The antibiofilm activity of ASL lectin after 24 hours of exposure is as shown in Fig 3b. At concentration of 500µg/ml, ASL lectin exhibited percentage reductions in biofilm adhesion as follows; no inhibition in *K. pneumoniae* (0%), 6.0% in *B. subtilis*, 22.3% in *S. aureus*, and 42.3% in *P. aeruginosa*. When tested at 100µg/ml, no inhibitory activity was detected in *K. pneumoniae*, *B. subtilis*, or *S. aureus*, while *P. aeruginosa* showed a 19% reduction. At the lowest concentration (10µg/ml), *K. pneumoniae* and *B. subtilis* exhibited no inhibition, whereas *S. aureus* and *P. aeruginosa* showed reductions of 2.4% and 20%, respectively.

Discussion:

Bacterial clumps known as biofilms are typically resistant to broad-spectrum antibiotics at normal therapeutic or even at higher dosages. Multi-drug resistance in biofilms, which cause the emergence of potentially fatal nosocomial infections, is on the rise and is becoming a major issue (5). From this study, the anti-adhesion effect of *T. africana* lectin extract indicates that it is limited at 4 hours but improved after 24 hours, without achieving complete inhibition. At 4 hours, the relatively higher reduction in biofilm observed in *S. aureus* compared to other isolates suggests that TAL lectin had a modest ability to interfere with early adhesion, whereas its negligible effect on *K. pneumoniae* and weak activity on *P. aeruginosa* reflect poor disruption of initial attachment. However, by 24 hours, the increased inhibition in *K. pneumoniae* and *B. subtilis* demonstrates that prolonged

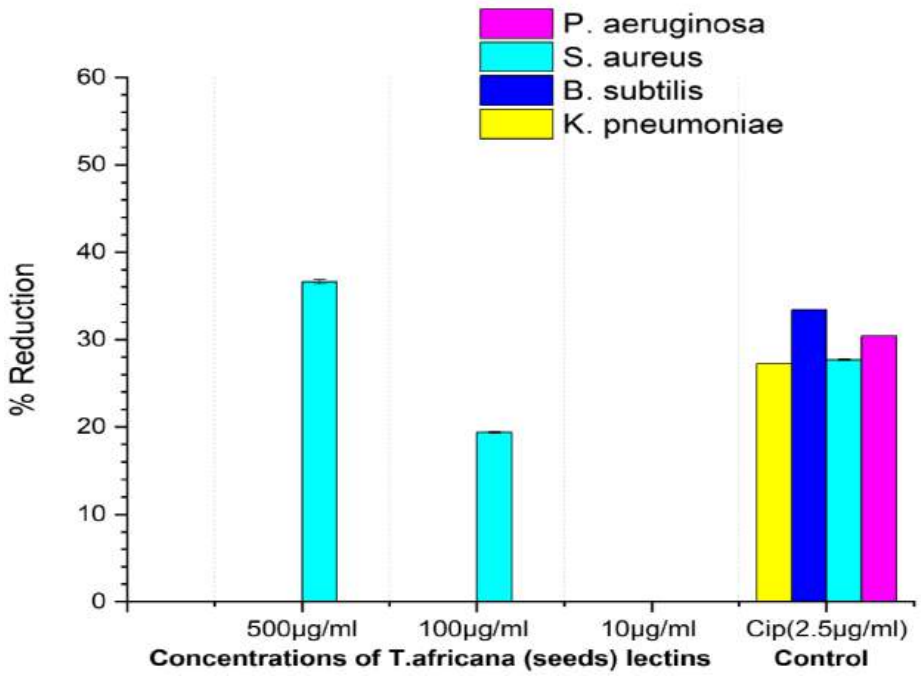


Fig 3a: Antiadhesion property of *Allium sativum* (bulbs) lectins at 4 hours exposure

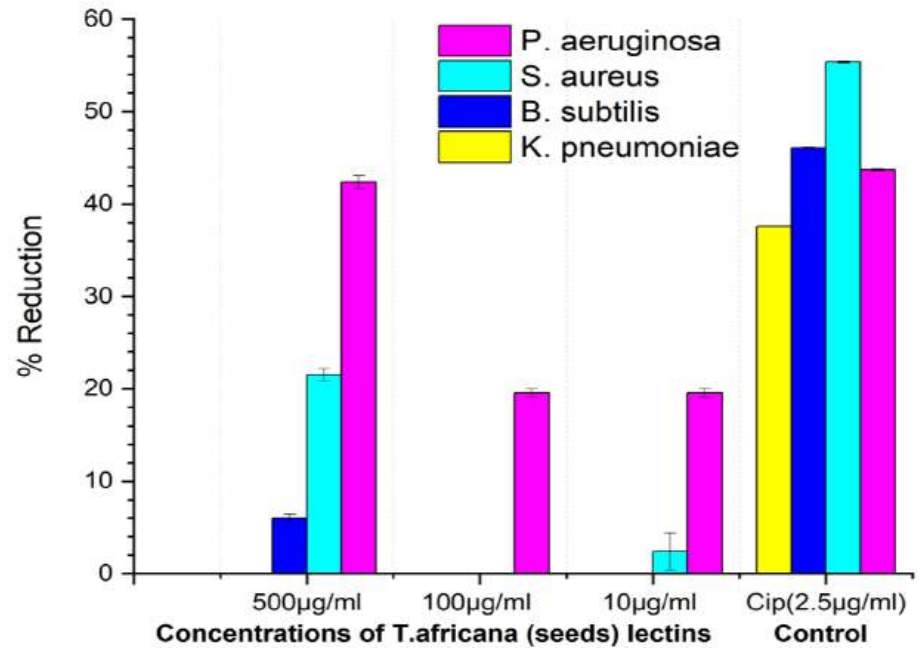


Fig 3b: Result showing antiadhesion property of *Allium sativum* (bulb) lectins at 24 hours exposure

exposure enhanced lectin interaction with bacterial surfaces, possibly affecting extracellular polymeric substance formation and cell signaling mechanisms essential for biofilm development (4,7,9).

Although this time-dependent improvement is partial, it supports the understanding that biofilm-associated bacteria possess adaptive resistance that limits complete inhibition (5). Furthermore, the differential response among the tested bacteria suggests that the activity of TAL is strongly influenced by bacterial cell

wall architecture. The relatively higher biofilm reduction in *S. aureus* and *B. subtilis* compared to *K. pneumoniae* and *P. aeruginosa* implies that structural accessibility of binding sites plays a role in lectin efficacy. In this context, the outer membrane of Gram-negative bacteria may restrict lectin penetration, whereas the exposed surface components in Gram-positive bacteria may facilitate stronger interactions, thereby explaining the observed variation in inhibition patterns.

Similarly, the results for *M. oleifera* lectin show a moderate but inconsistent anti-adhesion effect that varied with both time and bacterial species. At 4 hours, the strong inhibition observed in *P. aeruginosa* indicates that MOL lectin effectively interfered with early adhesion in this organism; however, the marked decline in activity at 24 hours suggests that this effect was not sustained as the biofilm matured. In contrast, the increased inhibition recorded for *K. pneumoniae* and *B. subtilis* at 24 hours compared to 4 hours indicates that prolonged exposure enhanced lectin–cell interactions in these species. Meanwhile, the relatively stable but moderate inhibition of *S. aureus* across both time points reflect partial susceptibility and adaptive resilience. Moreover, the inability of MOL lectin to completely prevent adhesion and the occasional reduction in effectiveness over time, reinforce the complexity of lectin–bacteria interactions and the adaptive capacity of biofilms. This observation aligns with earlier reports that disrupting initial adhesion is often more effective than targeting mature biofilms (22). Additionally, the variability in response among the isolates further supports the role of bacterial surface composition and biofilm-forming ability in determining lectin activity, consistent with the growing recognition of plant lectins as modulators of microbial attachment (23).

Meanwhile, the results for *A. sativum* lectin demonstrate comparatively weak and highly selective anti-adhesion activity. At 4 hours, the inhibition observed primarily in *S. aureus* indicates limited effectiveness in disrupting early adhesion in most organisms, while the absence of activity against *K. pneumoniae*, *B. subtilis*, and *P. aeruginosa* suggests minimal interaction with their cell surfaces. However, at 24 hours, moderate inhibition in *P. aeruginosa* indicates a delayed and organism-specific response, although the overall activity remained low. This restricted effectiveness contrasts with previous findings (24), highlighting variability in lectin activity across plant sources and bacterial targets. Nevertheless, the persistence of biofilm formation despite ASL lectin treatment emphasizes the resilience of biofilm-forming bacteria and the limited capacity of lectins to achieve complete inhibition. The weak performance of ciprofloxacin further supports this observation and underscores the challenge of biofilm-associated resistance (5). In addition, the possibility that lectins may enhance adhesion under certain conditions, as reported by Ofek et al., (25), provides a plausible explanation for the inconsistent inhibitory patterns, while the findings remain comparable to earlier reports on lectin activity against biofilms (26,27).

Conclusion:

The results of this study are significant because plant lectin may be used as a natural anti-adhesion agent to reduce early bacterial colonization, which is a crucial stage in developpment of infections.

Contributions of authors:

OUU and ESC were involved in all aspects of the study, including conceptualization, experimental design, data collection, analysis, and manuscript preparation; EK contributed to plant extraction as well as the preparation of stock solutions and standardization of test bacteria; NUV was responsible for plant extraction; AT and AAB handled statistical analysis; BRA was responsible for sourcing of plant materials; OB carried out the preparation of stock solutions and standardization of test bacteria; while EJO, OCS, EEC and EGO contributed to data interpretation and manuscript editing. All authors approved the manuscript submitted for publication.

Source of funding:

No funding was received for the study

Conflicts of interest:

Authors declare no conflicts of interest

References:

1. Vestby, L. K., Grønseth, T., Simm, R., and Nesse, L. L. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics. 2020; 9(2): 59. https://doi.org/10.3390/antibiotics9020059
2. Donlan, R. M., and Costerton, J. W. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002; 15 (2): 167–193. https://doi.org/10.1128/CMR.15.2.167-193.2002
3. Flemming, H. C., and Wingender, J. The biofilm matrix. Nat Rev Microbiol. 2010; 8 (9): 623–633. https://doi.org/10.1038/nrmicro2415
4. Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. Bacterial biofilms: From the natural environment to infectious diseases. Nat Rev Microbiol. 2004; 2(2): 95–108. https://doi.org/10.1038/nrmicro821

5. Mirghani, R., Saba, T., Khaliq, H., et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. AIMS Microbiol. 2022; 8 (3): 239–277. https://doi.org/10.3934/microbiol.2022019
6. Percival, S. L., Suleman, L., Vuotto, C., and Donelli, G. Healthcare-associated infections, medical devices & biofilms: Risk, tolerance and control. J Med Microbiol. 2015; 64 (4): 323–334. https://doi.org/10.1099/jmm.0.000032
7. Sharma, S., Mohler, J., Mahajan, D. S., Schwartz, S.A., Bruggemann, L., and Aalinkeel, R. Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms. 2023; 11 (6): 1614. https://doi.org/10.3390/microorganisms11061614
8. Eluu, S. C., Obayemi, J. D., Salifu, A. A., et al. *In-vivo* studies of targeted and localized cancer drug release from microporous poly-di-methyl-siloxane (PDMS) devices for the treatment of triple negative breast cancer. Sci Rep. 2024 ; 14 : 31. https://doi.org/10.1038/s41598-023-50656-6
9. Grari, O., Ezrari, S., El Yandouzi, I., et al. A comprehensive review on biofilm-associated infections: Mechanisms, diagnostic challenges, and innovative therapeutic strategies. The Microbe. 2025; 8: 100436. https://doi.org/10.1016/j.microb.2025.100436
10. Eluu, S. C., Oko, A. O., Eluu, K., Okoye, C. S., Onyekwere, U. U., and Omoniyi, O. A. Impact of *Newbouldia laevis* root extract on hematological parameters in rats: A comprehensive study on dosage-dependent effects and long-term dynamics. Nig Agric J. 2023; 54 (2): 324–329.
11. Eluu, S. C., Oko, A. O., Eluu, K., Onyekwere, U. U., and Okoye, C. S. Impact of *Newbouldia laevis* root extract on liver enzymes in rats. Nig Agric J. 2023; 54 (2): 259–264.
12. Komath, S., Marapakala, K., and Swamy, M. Beyond carbohydrate binding: New directions in plant lectin research. Org Biomol Chem. 2006; 4 (6): 973-988. https://doi.org/10.1039/b515446d
13. Ahmed, M. N., Jahan, R., Nissapatorn, V., Wilairatana, P., and Rahmatullah, M. Plant lectins as prospective antiviral biomolecules in the search for COVID-19 eradication strategies. Biomed Pharmacother. 2022; 146: 112507. https://doi.org/10.1016/j.biopha.2021.112507
14. Zhao, X., Yu, Z., and Ding, T. Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganisms. 2020; 8(3): 425. https://doi.org/10.3390/microorganisms8030425
15. Breitenbach Barroso Coelho, L. C., Marcelino Dos Santos Silva, P., Felix de Oliveira, W., et al. Lectins as antimicrobial agents. J Appl Microbiol. 2018; 125 (5): 1238–1252. https://doi.org/10.1111/jam.14055
16. Sandasi, M., Leonard, C. M., and Viljoen, A. M. The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes* Lett Appl Microbiol. 2010; 50 (1): 30–35. https://doi.org/10.1111/j.1472-765X.2009.02747.x
17. Ojimelukwe, P. C., and Ugwuona, F. U. The traditional and medicinal use of African breadfruit (*Treculia africana* Decne): An underutilized ethnic food of the Ibo tribe of South East, Nigeria. J Ethnic Foods. 2021; 8: 21. https://doi.org/10.1186/s42779-021-00097-1
18. Lakshmipriya, G., Kruthi, D., and Devarai, S. K. *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Sci Human Wellness. 2016; 5(2): 49–56. https://doi.org/10.1016/j.fshw.2016.04.001
19. Khatun, S., Khan, M. M. H., Ashraduzzaman, M., Pervin, F., Bari, L., and Absar, N. Antibacterial activity and cytotoxicity of three lectins purified from drumstick (*Moringa oleifera* LAM.) leaves. J Biol Sci. 2009; 17: 89–94. https://doi.org/10.3329/jbs.v17i0.7112
20. Laija, S. N., Mahesh, S., Smitha, L. S., and Remani, P. Isolation and partial characterization of two plant lectins. Curr Res J Biol Sci. 2010; 2 (4): 232–237.
21. Stepanovic, S., Vukovic, D., Dakic, I., Saric, B., and Svabic-Vlahovic, M. A modified microtitre plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods. 20000; 40: 175–179. doi: 10.1016/s0167-7012(00)00122-6.
22. Cerca, N., Martins, S., Pier, G. B., Oliveira, R., and Azered, J. The relationship between inhibition of bacterial adhesion to a solid surface by sub-MICs of antibiotics and subsequent development of a biofilm Res Microbiol. 2005; 156: 650–655. doi: 10.1016/j.resmic.2005.02.004
23. Ambrosi, M., Cameron, N. R., and Davis, G. B. Lectins: Tools for molecular understanding of the glycocode. Org Biomol Chem. 2005; 3: 1593–1608. https://doi.org/10.1039/B414350G
24. Oliveira, M. D. L., Andrade, C. A. S., Santos-Magalhães, N. S., et al. Purification of a lectin from *Eugenia uniflora* L. seeds and its potential antibacterial activity. Lett Appl Microbiol. 2008; 46 (4): 371–376. http://dx.doi.org/10.1111/j.1472-765X.2007.02319.x
25. Ofek, I., Hasty, D. L., and Sharon, N. Anti-adhesion therapy of bacterial disease: Prospects and problems. FEMS Immunol Med Microbiol. 2003; 38 (3): 181–191. doi: 10.1016/S0928-8244(03)00228-1
26. Teixeira, E. H., Napimoga, M. H., Carneiro, V. A., et al. *In vitro* inhibition of *Streptococci* binding to enamel acquired pellicle by plant lectins. J Appl Microbiol. 2006; 101: 111–116. doi: 10.1111/j.1365-2672.2006.02910.x
27. Wongkham, S., Boonsiri, P., Trisonthi, C., Simasathiansophon, S., Wongkham, C., and Atisook, K. Studies of lectins from Thai plants. J Sci Soc Thailand. 1995; 21: 27–36.

Ochekpe et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 371 - 377 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2462. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Prevalence of vaginal *Candida albicans* among women attending Federal Medical Centre, Makurdi, Nigeria

[1]Ochekpe, O. S., [1]Umeh, E. U., [1]Amali, O., [1]Ogbonna, I. O., [2]Adikwu, P., and *[2]Agbo, A. A.

[1]Federal University of Agriculture, Makurdi, Benue State, Nigeria
[2]Federal University of Health Sciences, Otukpo, Benue State, Nigeria
*Correspondence to: ustynameh@gmail.com

Abstract:

Background: *Candida albicans* accounts for the most common type of opportunistic fungal infection affecting human heath globally, with more than a billion cases annually. The prevalence is reportedly high in resource-limited settings. The aim of this study is to determine the prevalence of vaginal *C. albicans* among women attending Federal Medical Centre, Makurdi, Nigeria.
Methodology: Vaginal swab samples were collected from a total of 698 women attending both the Outpatient Department (OPD) and the HIV clinic at Federal Medical Centre, Makurdi. *Candida albicans* was isolated on Sabouraud Dextrose Agar, and confirmed by the germ tube test and growth on CHROMAgar plate.
Results: The isolation rate of *C. albicans* among the 698 selected women was 49.0% (n=342). The highest prevalence was among women aged 41–50 years (90.7%, n=78/86) and widowed (58.0%, n=29/50). Significant associations were observed between *C. albicans* prevalence and variables such as location (χ^2=15.497, p<0.0001), age (χ^2=207.35, p<0.0001), marital status (χ^2=7.833, p=0.0199), occupation (χ^2=14.668, p=0.0054), and HIV status (χ^2=6.578, p=0.0373)
Conclusion: Public health education programmes should be implemented to raise awareness about *Candida albicans* infections.

Keywords: *Candida albicans*, vagina, HIV status, occupation, age, gender, location

Received Oct 8, 2025; Revised Mar 22, 2026; Accepted Mar 23, 2026

Prévalence des vaginales *Candida albicans* chez les femmes consultant le Centre médical fédéral de Makurdi, au Nigéria

[1]Ochekpe, O. S., [1]Umeh, E. U., [1]Amali, O., [1]Ogbonna, I. O., [2]Adikwu, P., et *[2]Agbo, A. A.

[1]Université Fédérale d'Agriculture, Makurdi, État de Benue, Nigéria
[2]Université Fédérale des Sciences de la Santé, Otukpo, État de Benue, Nigéria
*Correspondance à: ustynameh@gmail.com

Résumé:

Contexte: *Candida albicans* représente le type d'infection fongique opportuniste le plus fréquent à l'échelle mondiale, avec plus d'un milliard de cas par an. Sa prévalence est particulièrement élevée dans les pays à ressources limitées. Cette étude vise à déterminer la prévalence des candidoses vaginales à *C. albicans* chez les femmes consultant au Centre Médical Fédéral de Makurdi, au Nigéria.
Méthodologie: Des prélèvements vaginaux ont été effectués chez 698 femmes consultant au service de consultations externes et à la clinique VIH du Centre médical fédéral de Makurdi. *Candida albicans* a été isolé sur gélose Sabouraud dextrose et confirmé par le test de germination et la culture sur milieu CHROMAgar.
Résultats: Le taux d'isolement de *C. albicans* parmi les 698 femmes sélectionnées était de 49,0% (n=342). La prévalence la plus élevée a été observée chez les femmes âgées de 41 à 50 ans (90,7%, n=78/86) et chez les veuves (58,0%, n=29/50). Des associations significatives ont été observées entre la prévalence de *C. albicans* et des variables telles que la localisation (x^2=15,497, p<0,0001), l'âge (x^2=207,35, p<0,0001), la situation matrimoniale

(x^2=7,833, p=0,0199), la profession (x^2=14,668, p=0,0054) et le statut VIH (x^2=6,578, p=0,0373). **Conclusion:** Des programmes d'éducation à la santé publique devraient être mis en œuvre afin de sensibiliser la population aux infections à *Candida albicans*.

Mots-clés: *Candida albicans*, vagin, statut VIH, profession, âge, sexe, lieu de résidence.

Introduction:

Candidiasis is an infection caused by the overgrowth of pathogenic yeast from the *Candida* genus (1). Its prevalence accounts for the most common type of opportunistic fungal infection affecting human heath globally, with more than a billion cases annually (2,3). Typically, yeast can live harmlessly on the host's mucosal tissues, such as in the oral cavity, gastrointestinal mucosa and vaginal mucosa, unless balance is disrupted (4,5). The immunosuppressed, elderly population and palliative patients are highly susceptible to *Candida* infections (6). Oral candidiasis results in local oral pain and discomfort, enhanced oral dryness, loss of taste and aversion to food and may lead to secondary complications (7).

The distribution of *Candida* species in women can vary depending on geographical location, population demographics and other factors (8). *Candida* species are commonly found in the genital tract of women. Historically, *Candida albicans* has been the most prevalent *Candida* species in vaginal infections (9) and is a natural inhabitant of the human body, including the genital tract (10). It is typically responsible for uncomplicated vulvovaginal candidiasis (VVC) (2,10,11). It is important to note that the distribution of *Candida* species can change over time due to factors such as antimicrobial use, changes in sexual practices and the emergence of antifungal resistance (8).

Accurate identification of the causative *Candida* species is crucial for effective treatment, as different species may exhibit varying susceptibilities to antifungal medications (12). Additionally, the presence of non-albicans *Candida* species, which may be less responsive to standard treatments, underscores the importance of proper diagnosis and tailored therapy in the management of vaginal candidiasis (13, 14). The study was aimed at determining the prevalence of *Candida* species from women attending Federal Medical Centre, Makurdi, Nigeria

Materials and method:

Study area:

The study was carried out in Makurdi. Makurdi is the capital of Benue State. Makurdi lies between latitude 7° 43' 50" N and longitude 8° 32' 10" E. The town is divided into two major blocks by River Benue, hence, the North and South bank. Makurdi is inhabited predominantly by the Tiv and Jukun people. Makurdi being the State capital, has become a cosmopolitan city with people of different tribes as Idoma, Igede, Agatu, Etulo, Igbo, Hausa and Igala (15). Makurdi have an estimated population of 500,797 with a landmass of 16km radius (16).

Study participants:

The study participants include both symptomatic and asymptomatic women attending outpatient department (OPD) and HIV clinic of the Federal Medical Centre, Makurdi. A total of 698 women were enrolled in the study. Participants included both symptomatic and asymptomatic women.

Symptomatic participants were those presenting with clinical features suggestive of vulvovaginal candidiasis such as vaginal itching, discharge, irritation, and discomfort while asym ptomatic participants showed no obvious signs of infection due *Candida albicans* among different clinical categories.

Sample size determination:

Sample size was determined using this formula (17); $S= X^2 NP (1-P)/d^2 (N-1) + X^2P (1-P)$, where S= Sample size being sought, X^2 = table value for Chi-square at 1 degree of freedom at the desired alpha level (0.05=3.84), N = Population size, P = the population proportion (usually 0.05 as this provides the maximum sample size) and d = degree of accuracy desired, expressed as a proportion (usually 0.05).

Inclusion and exclusion criteria:

The study included women within the reproductive age group, who were symptomatic or asymptomatic for vaginal infections, and who gave consent to participate in the study. The following individuals were excluded from the study; women below the age of childbearing and women who did not provide consent.

Sampling technique:

A combination of purposive and random sampling techniques was employed. Purposive sampling was initially used to select eligible participants attending the OPD and HIV clinic based on the study criteria. Thereafter, random sampling was applied to ensure that participants were selected without bias, thereby improving the representativeness of the study population.

Data and sample collection:

Vaginal samples were collected from 698 women attending OPD and HIV clinic at Federal Medical Centre, Makurdi. The samples collected were appropriately labeled and were transported to the laboratory for inoculation and identification. In addition, data were collected directly from participants using a structured questionnaire.

The data included socio-demographic data (age, marital status, educational level, occupation), clinical data (presence or absence of symptom such as itching, discharge, irritation) and potential risk factors (antibiotic use, contraceptive use, hygiene practices, underlying disease conditions such as HIV).

Microbiological analysis and identification of *Candida:*

The samples were inoculated onto Sabouraud Dextrose agar and incubated at 37^0C for 24h. The colonies obtained were purified by sub-culturing onto ChromAgar *Candida* medium and incubated at 37^0C for 24 hours. The colonies obtained were sub-cultured repeatedly to obtain pure isolates**.** The presumptive isolates were identified using Gram stain and germ tube test as described by (18).

Statistical analysis:

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 29.0. Pearson's Chi-square test was used to determine associations between variables at 95% confidence level. A *p* value less than or equal to 0.05 was considered to be indicative of a statistically significant relationship.

Results:

The isolation rate of *Candida albicans* in the study population was 49.0% (n=342/698), while 51.0% (n=356/698) were negative. The HIV clinic had a higher prevalence rate (61.0%, n=122/200) than the outpatient department (44.2%, n=220/498). Isolation rate of *C. albicans* with respect to location is as presented in Fig 1. The difference in isolation rate of *C. albicans* with respect to location was statistically significant (χ^2=15.497, p<0.0001).

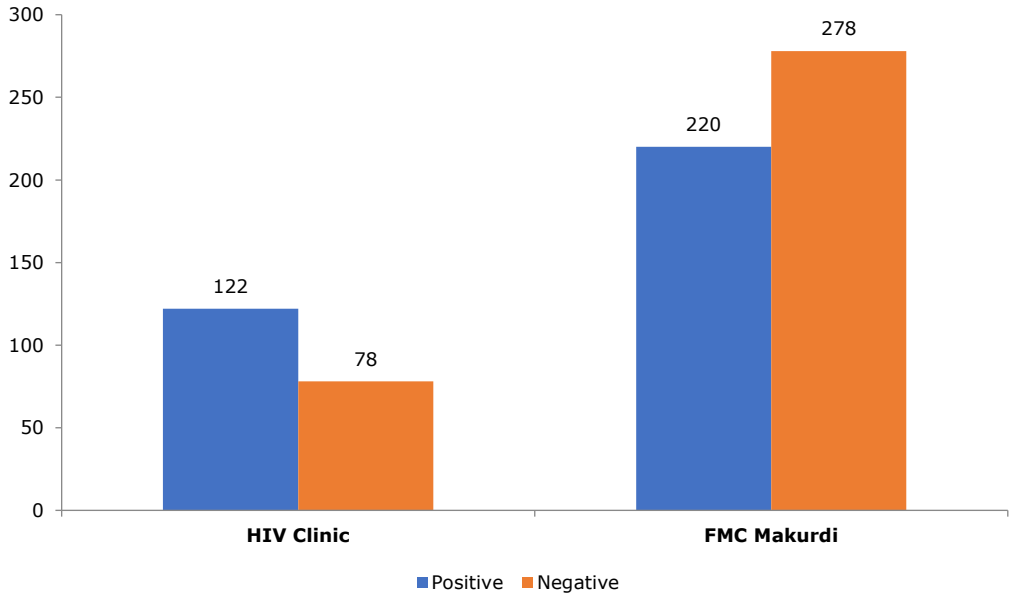


Fig 1: Frequency of *Candida albicans* with respect to location

Table 1: Bivariate analysis of prevalence of *Candida albicans* and socio-demographic and risk factors among women attending outpatient department and HIV clinic of Federal Medical Centre, Makurdi, Nigeria

Variables	No positive (%)	No negative (%)	Total	x^2	*p* value
Age group (years)					
≤20	27 (72.9)	10 (27.1)	37	207.35	<0.0001*
21-30	43 (20.7)	165 (79.3)	208		
31-40	106 (70.7)	44 (29.3)	150		
41-50	78 (90.7)	8 (9.3)	86		
51-60	76 (57.1)	57 (42.9)	133		
>60	12 (14.3)	72 (85.7)	84		
Marital status					
Single	90 (41.5)	127 (58.5)	217	7.833	0.0199*
Married	223 (51.7)	208 (48.3)	431		
Widowed	29 (58.0)	21 (42.0)	50		
Educational status					
Primary	21 (41.2)	30 (58.8)	51	2.854	0.4148
Secondary	114 (50.4)	112 (49.6)	226		
Tertiary	202 (48.8)	212 (51.2)	414		
None	5 (71.4)	2 (28.6)	7		
Occupation					
Civil service	138 (54.5)	115 (45.5)	253	14.668	0.0054*
Trading	137 (50.7)	133 (49.3)	270		
Farming	3 (75.0)	1 (25.0)	4		
Students	35 (34.3)	67 (65.7)	102		
Housewife	29 (42.0)	40 (58.0)	69		
Underwear type					
Pant	176 (49.7)	178 (50.3)	354	0.4359	0.8041
Short	85 (49.7)	86 (50.3)	171		
Tight	81 (46.8)	92 (53.2)	173		
HIV status					
Positive < 5 years	46 (52.9)	41 (47.1)	87	6.578	0.0373*
Positive ≥ 5 years	67 (58.8)	47 (41.2)	114		
Negative	229 (46.1)	268 (53.9)	497		
Symptoms					
Itching	49 (57.6)	36 (42.4)	85	3.798	0.2841
Discharge	5 (35.7)	9 (64.3)	14		
Itching + Discharge	123 (47.5)	136 (52.5)	259		
No symptoms	165 (48.5)	175 (51.5)	340		
Drug use history					
Prescribed drugs	154 (48.1)	166 (51.9)	320	2.875	0.4113
Over-the-counter drug	45 (56.3)	35 (43.7)	80		
Contraceptive drugs	19 (41.3)	27 (58.7)	46		
No drugs	124 (49.2)	128 (50.8)	252		
Total	**342 (49.0)**	**356 (51.0)**	**698**		

x^2 = Chi square; * = statistically significant at $p<0.05$

The prevalence of *C. albicans* across the socio-demographic and risk factors is shown in Table 1. The highest prevalence was in the age group 41-50 years with 90.7% (n=78/86) while the age group >60 years had the lowest prevalence of 14.3% (n=12/84). This difference in prevalence was statistically significant (χ^2=207.4, p<0.0001). The prevalence of *C. albicans* with respect to marital status showed that women who are widowed had the highest prevalence (58.0%, n=29/50) while the single women had the lowest prevalence (41.5%, n=90/217). The difference in prevalence was statistically significant (χ^2=7.833, p=0.0199).

The isolation rate of *C. albicans* with respect to educational status showed that women with no education had the highest isolation rate (71.4%, n=5/7) while those with primary education had the least prevalence (41.2%, n=21/51). The difference in isolation rate is, however, not statistically significant (χ^2=2.834, p=0.415). The prevalence of *C. albicans* with respect to occupation showed that women who are farmers had the highest rate of isolation (75.0%, n=3/4), followed by civil servants (54.5%, n=138/253), and traders (50.7%, n=137/270). In contrast, students had the lowest isolation rate of 34.3% (n=35/102). The

difference in isolation rates across the occupational groups was statistically significant (χ^2= 14.668, *p*=0.0054).

The prevalence of *C. albicans* with respect to nature of underwear showed that the highest isolation rate was among women who wear pants (49.7%, n=176/354) and shorts (49.7%, n=85/171) while the least isolation was among women who wear tights (46.8%, n=81/173). The difference in isolation rate with respect to underwear was not statistically significant (χ^2=0.4359, *p*=0.804). The isolation rate of *C. albicans* in relation to HIV status showed that those with HIV infection for ≥5 years had the highest isolation rate of 58.5% (n=67/114), followed by individuals with HIV infection for <5 years with 52.9% (n=46/87). HIV-negative individuals had the lowest isolation rate of 46.1% (n=229/497). The difference in isolation rates with respect to HIV status was statistically significant (χ^2=6.578, *p*= 0.0373).

The prevalence of *C. albicans* in relation to reported symptoms showed that highest isolation rate was observed among individuals experiencing itching (57.6%, n=49/85), while those with discharge had the lowest rate (35.7%, n=5/14). However, the difference in isolation rate across symptom types was not statistically significant (χ^2=3.798, *p*=0.284).

The prevalence of *C. albicans* in relation to drug use showed that patients who used over-the-counter drugs had the highest isolation rate (56.3%, n=45/80), while those who used contraceptive pills had the lowest isolation rate at 41.3% (n=19/46). The difference in isolation rate was, however, not statistically significant (χ^2=2.875, *p*=0.4113).

Discussion:

The study was aimed at determining the prevalence of vaginal *C. albicans* among women attending the Federal Medical Centre, Makurdi, Benue State. The findings revealed *C. albicans* isolation rate of 49.0%, indicating a significant public health challenge in the study area. The prevalence observed in this study is comparable to a previous study conducted in Owerri, Imo State, Nigeria, which reported rate as high as 57% among women (19). Oral candidiasis prevalence of approximately 20.5% has been reported among patients with haematological malignancies (20), while vulvovaginal candidiasis prevalence of up to 50.2% has been documented in Egypt (21). However, some studies focused primarily on antifungal resistance and molecular characteristics rather than prevalence (22, 23). The variations in rates could be attributed to differences in population characteristics, healthcare practices, or local antibiotic and contraceptive usage patterns. The public health implication of the high prevalence of *C. albicans* observed in this study suggests the need for heightened awareness and preventive measures in the region.

According to reports of World Health Organization, the majority of *C. albicans* cases occur in Asia, Africa and Latin America where abuse of antibiotics and oral contraceptives are highly prevalent (24). Patients that attended the HIV clinic had a higher prevalence of *C. albicans* compared to patients at the OPD. This disparity is likely attributable to immunosuppressive effects of HIV/AIDS, which predispose individuals to opportunistic infections such as candidiasis. These results emphasize the importance of targeted interventions, particularly among immunocompromised populations, to lessen the spread and impact of *C. albicans*. Regular screening, judicious use of antibiotics, and public health campaigns emphasizing preventive measures could play a crucial role in reducing the burden of candidiasis in the study area.

Patients in the age group 41-50 years had the highest prevalence of 90% (n=78/86), followed by age group <20 years (72.9%, n=27 /37), 31-40 years (70.7%, n=106/150) and least prevalence in >60 years (14.3%, n=12/ 84). This pattern of indicates that infection is not strictly confined to younger reproductive-age women but may extend into late reproductive and perimenopausal age groups. This finding is partially consistent with the observations reported in Edo state that higher burden of infection among sexually active age groups, although their peak prevalence was observed in younger women. The similarity lies in the fact that sexually active and hormonally dynamic age groups remain the most affected, even though the exact peak age differs. The higher prevalence in the 41-50 age group in this study may reflect prolonged exposure to risk factor, including repeated antimicrobial use, chronic underlying conditions, hormonal fluctuations associated with the perimenopausal transition (22). Similarly, the findings align with previous reports which indicate that middle-age women maintain substantial susceptibility to *Candida* infections, particularly in the predisposing factors such as diabetes, antibiotic misuse, and immunological changes. This supports the observation that susceptibility may extend beyond peak reproductive years, especially in populations experiencing high antimicrobial pressure or comorbidities (19).

Furthermore, the observed pattern is consistent with studies demonstrating that age-

related physiological changes, including oestrogen levels, alterations in vaginal microbiota, and reduced immune efficiency, significantly influence infection dynamics. These factors may explain the elevated prevalence observed in the 41-50 years age group, as this stage is often characterized by hormonal instability, which disrupts normal vaginal flora and promotes *Candida* overgrowth (25). The relatively high prevalence among individuals <20 years may be attributed to poor hygiene practices, immature immune responses, and early sexual exposure, while the low prevalence in those >60 years could be due to reduced sexual activity and lower estrogen-dependent glycogen availability, which claims *Candida* proliferation.

The findings of this study revealed several important demographic factors associated with the prevalence of *C. albicans*. Concerning marital status, the widow and married groups had significantly higher *C. albicans* isolation rate of 58.0% and 51.7% respectively, compared to single women with 41.5% (p=0.0199). This observation is consistent with previous reports, although variations in prevalence have been documented across different populations. For instance, a study conducted in Owerri reported a prevalence of 53.34%, while another study in Kano documented a prevalence of 45.7%, indicating a relatively high burden of infection among women of reproductive age (19,26). Similar trends have also been reported in other settings, although the magnitude of prevalence varies depending on geographical location, study population, and methodological differences.

Patients who frequently patronized over the counter medications had the highest rate of isolation rates of *C. albicans*. This is consistent with previous reports indicating that the misuse and overuse of antibiotics, as well as the use of oral contraceptives, are significant predisposing factors for *Candida* infection (19,26,27).

Conclusion:

In conclusion, the findings of the study revealed a high *Candida albicans* isolation rate of 49.0%, indicating a significant public health challenge from vaginal candidiasis. The rate of *C. albicans* infection showed preponderance of HIV clinic over OPD attendees. The study has also highlighted a statistically significant association between prevalence of *C. albicans* and demographic factors such as the age groups, marital status, location and occupation in the study area, with the highest prevalence occurring among women aged 41-50 years and among widowed and married women. However, no statistically significant association was observed between *C. albicans* isolation rate and educational status, type of underwear, symptoms and history of drug use. A synergistic (combination) therapeutic approach should be taken into consideration in the treatment of infection caused by *Candida albicans*.

Acknowledgements:

The authors acknowledge Prof. (Mrs.) Umeh Ebelle Uchenna and Prof. Amali Okwoli for their guidance. The authors also appreciate the support of their colleagues and the enabling environment provided by the University.

Contributions of authors:

OOS, UEU and AO conceived and designed the study; OOS and AP carried out sample collection and laboratory analyses; AAA and AP performed data analysis and interpretation; OOS drafted the manuscript; UEU, AO and OIO critically revised the manuscript for important intellectual content; and AAA served as the corresponding author. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Source of funding:

No funding was received for the study

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Bhattacharya, S., Sae-Tia, S., and Fries, B. C. Candidiasis and mechanisms of antifungal resistance. Antibiotics. 2020; 9: 312. doi: 10.3390/antibiotics9060312
2. Bunetel, L., Tamanai-Shacoori, Z., Martin, B., Autier, B., Guiller, A., and Bonnaure-Mallet, M. Interactions between oral commensal *Candida* and oral bacterial communities in immunocompromised and healthy children. J Med Mycol. 2019; 29 (3): 223–232. doi: 10.1016/j.mycmed.2019.06.004
3. Fisher, M. C., Gow, N. A. R., and Gurr, S. J. Tackling emerging fungal threats to animal health, food security and ecosystem resilience. Philos Trans R Soc B Biol Sci. 2016; 371 (1709): 20160332. doi: 10.1098/rstb.2016.0332.
4. Kourkoumpetis, T. K., Velmahos, G. C., Ziakas, P. D., et al. The effect of cumulative length of hospital stays on antifungal resistance of *Candida* strains. Mycopathologia. 2011; 171: 85–91. doi: 10.1007/s11046-010-9369-3.
5. Dąbrowska, M., Sienkiewicz, M., Kwiatkowski, P., zielińska-Bliźniewska, H., and Dąbrowski, M. Diagnosis and treatment of mucosa *Candida* spp. infections—A review article. Ann Univ Mariae Curie Sklodowska Sect C. 2018; LXXIII (1): 61–68. doi: 10.17951/c.2018.73.1.61-68

6. Gonsalves, W. C., Wrightson, A. S., and Henry, R. G. Common oral conditions in older persons. Am Fam Physician. 2008;78(7):845–852.
7. Lalla, R. V., Latortue, M. C., Hong, C. H., et al. A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer. 2010; 18: 985–992. doi: 10.1007/s00520-010-0892-z
8. Jayachandran, A. L., Katragadda, R., Thyagarajan, R., Vajravelu, L., Manikesi, S., and Kaliappan, S. Candidiasis among cancer patients attending a tertiary care hospital in Chennai, South India. Can J Infect Dis Med Microbiol. 2016; 2016: 875–881. doi: 10.1155/2016/8758461
9. Ramla, S., Sharma, V., and Patel, M. Influence of cancer treatment on *Candida albicans*. Support Care Cancer. 2016; 24 (6): 2429–2436. doi: 10.1007/s00520-015-3035-8.
10. Lee, C. H., Lin, C., Ho, C. L., and Lin, J. C. Primary fungal prophylaxis in hematological malignancy. Antimicrob Agents Chemother. 2018; 62 (8): e00355-18. doi: 10.1128/AAC.00355-18
11. Lotfali, E., Mardani, M., Abolghasemi, S., et al. Isolation of *Candida africana* in oral candidiasis: First report among cancer patients in Iran. Curr Med Mycol. 2020; 6: 1076–1083. doi: 10.18502/CMM.6.2.2695
12. Ziba, A. N., Shirin, F. M. F., Mahtab, A. K., et al. Molecular identification of non-albicans *Candida*. Iran Biomed J. 2018;22(1):33–41. doi: 10.22034/ibj.22.1.33
13. Nejat, Z. A., Farahyar, N., Falahati, M., et al. Molecular identification and antifungal susceptibility of non-albicans *Candida*. Iran Biomed J. 2018; 22 (1): 33–41. doi: 10.22034/ibj.22.1.33.
14. Mohammadi, F., Hemmat, N., Bajalan, Z., and Javadi, A. Analysis of biofilm-related genes and antifungal susceptibility pattern. Biomed Res Int. 2021; 2021: 237–342. doi: 10.1155/2021/5598907.
15. Benue State Ministry of Lands, Survey and Solid Minerals 2015
16. National Population Commission. Federal Republic of Nigeria Official Gazette. 2009; 96 (2): 12–13.
17. Rosie, C. Statistics: An introduction to sample size calculations. 2006.
18. Ochei, J. O., and Kolhatkar, A. Medical laboratory science and practice. New Delhi: Tata McGraw-Hill; 2008.
19. Nwachukwu, M. O., Azorji, J. N., Onyebuagu, P. C., Adjeroh, L. A., and Nmezi, S. N. Prevalence of *Candida albicans* among female patients in Owerri. Int J Trop Dis Health. 2020;41(9):1–8. doi: 10.9734/ijtdh/2020/v41i930313
20. Mardani, M., Abolghasemi, S., Darvishnia, D., et al. Oral candidiasis in hematological malignancy patients. Jundishapur J Microbiol. 2020; 13 (8): e103290. doi: 10.5812/jjm.103290
21. ElFeky, D. S., Gohar, N. M., El-Seidi, E. M., Ezzat, M. M., and AboElew, S. H. Species identification and antifungal susceptibility pattern of *Candida* isolates in cases of vulvovaginal candidiasis. Alex J Med. 2016; 52: 269–277. doi: 10.1016/j.ajme.2015.10.001
22. Odiba, A. S., Durojaye, O. A., Ezeonu, I. M., Mgbeahuruike, A. C., and Nwanguma, B. C. Mutational signatures in ERG11 gene of fluconazole-resistant *Candida albicans*. Infect Drug Resist. 2022; 15: 3111–3133. doi: 10.2147/IDR.S360973
23. Jabeen, G., Naz, S. A., Rangel, D. E. N., Jabeen, N., Shafique, M., and Yasmeen, K. *In-vitro* evaluation of virulence markers and antifungal resistance of clinical *Candida albicans* strains isolated from Karachi, Pakistan. Fungal Biol. 2023; 127: 1241–1249.doi:10.1016/j.funbio.2023.04.003
24. World Health Organization. WHO fungal priority pathogens list. Geneva: WHO; 2022.
25. Shi, Y., Zhu, Y., Fan, S., Liu, X., Liang, Y., and Shan, Y. Molecular identification and antifungal susceptibility of yeast. BMC Infect Dis. 2020; 20: 287. doi: 10.1186/s12879-020-04985-w
26. Sule, H., Kumurya, A. S., and Muhammad, B. K. Candidiasis among single and married women in Kano. World J Biol Pharm Health Sci. 2020; 2 (3): 01–07.doi:10.30574/wjbphs.2020.2.3.0031
27. Agbedo, E. S., Osumah, O. R., Woghiren, E. P., and Omusi, I. P. Prevalence of *Candida albicans* among pregnant and non-pregnant women attending a medical facility in Oredo, Edo State, Nigeria. J Appl Sci Environ Manage. 2023; 7 (1): 345-358. doi:10.4314/jasem.v27i1.15

Salim & Nabila. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 378 - 384 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2484. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Prevalence of pulmonary mycoses among patients with suspected pulmonary tuberculosis at the Infectious Disease Hospital, Kano, Nigeria

*Salim, A. B., and Nabila, M. I.

Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria
*Correspondence to: ahmadbsalim@gmail.com; +2348033731917

Abstract:

Background: Opportunistic fungal infections have been implicated among patients with tuberculosis (TB). Coexistence of pulmonary mycoses and pulmonary TB is of public health concern as they exhibit similar clinical manifestations among infected patients, which contributes to increased misdiagnosis and makes management difficult, especially in TB-prone regions like Kano, Nigeria. Thus, this study was aimed at determining the prevalence of pulmonary mycoses among patients with suspected pulmonary TB attending the Infectious Disease Hospital (IDH), Kano State, Nigeria.
Methodology: Sputum samples were collected from a total of 155 patients with clinically suspected TB. The samples were analysed for the detection of *Mycobacterium tuberculosis* (MTB) using GeneXpert in line with the National Tuberculosis and Leprosy Control Program (NTBLCP) standard operating procedures and for the isolation and identification of pulmonary mycoses using cultural methods on Sabouraud Dextrose Agar (SDA) and microscopy techniques including lactophenol cotton blue stain, Gram stain, 10% KOH wet mount and germ tube tests.
Results: Of the total of 155 study participants recruited into the study, 67 (43.2%) were males and 88 (56.8%) were females. Of these, 88 (56.8%) and 31 (20.0%) were positive for pulmonary mycoses by culture and PTB by GeneXpert assay, respectively, while 9.0% (n=16) had pulmonary mycoses-PTB co-infection**.** *Aspergillus* spp was the most frequent fungus (n=76, 86.4%) identified while *Cryptococcus neoformans* and *Histoplasma capsulatum* were the least frequent, each with 2.3% (n=2).
Conclusion: The high rate of fungal infection (56.8%) among patients with suspected pulmonary TB is a significant public health challenge. There is need for routine screening of all patients with suspected TB for pulmonary mycoses, especially in high TB-burden regions, as this will contribute to improved management and reduction in morbidity.

Keywords: Pulmonary mycoses, pulmonary tuberculosis, GeneXpert, fungal culture, co-infection

Received Jan 7, 2026; Revised Apr 6, 2026; Accepted Apr 8, 2026

Prévalence des mycoses pulmonaires chez les patients présentant une suspicion de tuberculose pulmonaire à l'hôpital des maladies infectieuses de Kano, au Nigéria

*Salim, A. B., et Nabila, M. I.

Département de microbiologie, Faculté des sciences de la vie, Université Bayero, Kano, PMB 3011, Kano, Nigéria
*Correspondance à: ahmadbsalim@gmail.com; +2348033731917

Résumé:

Contexte: Des infections fongiques opportunistes ont été observées chez les patients atteints de tuberculose (TB).

La coexistence de mycoses pulmonaires et de tuberculose pulmonaire constitue un problème de santé publique, car ces deux affections présentent des manifestations cliniques similaires chez les patients infectés, ce qui contribue à l'augmentation des erreurs de diagnostic et complique la prise en charge, notamment dans les régions à forte prévalence de tuberculose comme Kano, au Nigéria. Cette étude visait donc à déterminer la prévalence des mycoses pulmonaires chez les patients présentant une suspicion de tuberculose pulmonaire et consultant à l'hôpital des maladies infectieuses (IDH) de l'État de Kano, au Nigéria.

Méthodologie: Des échantillons d'expectorations ont été prélevés chez 155 patients présentant une suspicion clinique de tuberculose. Ces échantillons ont été analysés pour la détection de *Mycobacterium tuberculosis* (MTB) par la technique GeneXpert, conformément aux procédures opératoires standard du Programme national de lutte contre la tuberculose et la lèpre (NTBLCP), et pour l'isolement et l'identification des mycoses pulmonaires par culture sur gélose Sabouraud dextrose (SDA) et par microscopie (coloration au bleu coton lactophénol, coloration de Gram, examen microscopique direct après coloration au KOH à 10% et test de germination).

Résultats: Sur les 155 participants inclus dans l'étude, 67 (43,2%) étaient des hommes et 88 (56,8%) des femmes. Parmi ces patients, 88 (56,8%) et 31 (20,0%) étaient positifs respectivement pour les mycoses pulmonaires (culture) et la tuberculose pulmonaire (test GeneXpert), tandis que 9,0% (n=16) présentaient une co-infection mycoses pulmonaires-tuberculose pulmonaire. *Aspergillus* spp était le champignon le plus fréquemment identifié (n=76, 86,4%), tandis que *Cryptococcus neoformans* et *Histoplasma capsulatum* étaient les moins fréquents, chacun représentant 2,3% des cas (n=2).

Conclusion: La forte prévalence d'infections fongiques (56,8%) chez les patients présentant une suspicion de tuberculose pulmonaire constitue un enjeu majeur de santé publique. Un dépistage systématique des mycoses pulmonaires chez tous les patients suspects de tuberculose est nécessaire, en particulier dans les régions à forte prévalence de tuberculose, afin d'améliorer la prise en charge et de réduire la morbidité.

Mots-clés: Mycoses pulmonaires, tuberculose pulmonaire, GeneXpert, culture fongique, co-infection

Introduction:

Pulmonary mycoses are fungal infections of the lungs that resemble pulmonary tuberculosis (PTB). Opportunistic fungal infections have been implicated in the rise of pulmonary mycoses among patients with TB, which poses a diagnostic challenge. Many other factors are also implicated in the increase of these fungal infections, but the most common causes remain PTB, HIV/AIDS, and extensive use of immunosuppressive drugs (1). Extensive use of antibiotics and steroids has also resulted in increasing trends of these fungal infections globally.

In the environment, there is a significant number of fungi and their spores which colonize the lung parenchyma by various modes such as inhalation, blood route, or by reactivation of a dormant infection and as such pose a major challenge in patients with PTB (2). Common fungal pathogens associated with pulmonary mycoses isolated from patients with PTB, include *Aspergillus* spp such as *Aspergillus niger* and *Aspergillus fumigatus*, *Histoplasma capsulatum* and *Cryptococcus neoformans* (3).

Tuberculosis is a potentially serious chronic lung disease that is caused by *Mycobacterium tuberculosis* and characterized by injury to the lung parenchyma with a granulomatous lesion along with epithelioid macrophages and Langhans giant cells with fibroblasts and collagenous tissue, as well as hallmark caseous necrosis in the centre (4). There have been studies that suggest that the chronic nature of tuberculosis tends to be more lethal when it is associated with annexation by parasitic fungi, as not only does it make the TB more virulent, but it also adds to various complications and even death (5).

Coexistence of pulmonary mycoses and PTB contribute to increased misdiagnosis, mismanagement and mistreatment by physicians because they do not show specific clinical manifestations, and as such leads to high morbidity and considerable mortality (6). Identification and characterization of fungal pulmonary pathogens among TB suspects would be of immense importance because it will lead to improved proper diagnosis where pulmonary mycoses are diagnosed and/or excluded. Thus, proper medications and managements can be adjusted accordingly, enhancing adherence and good treatment outcomes, especially in TB-prone regions such as Kano, Nigeria.

This study therefore was aimed at determining the prevalence of pulmonary mycoses among patients with suspected PTB attending the Infectious Disease Hospital (IDH), Kano, Nigeria.

Materials and method:

Study area:

The study was conducted at the Infectious Diseases Hospital (IDH), Kano, Nigeria. Located along France road within Kano metropolis, IDH is the main hospital that is designated by Kano State Government for treatment of infectious diseases such as TB, HIV/AIDS malaria, meningitis etc.

Kano city is located between longitude 8.500°E and latitude 11.500°N. It consists pri-

marily of Sudan savanna type vegetation, with mean annual rainfall of 800-900 mm, and temperature that ranges between 25–40°C. The hospital receives patients from the whole of northern parts of Nigeria, and serves as referral centre for TB and HIV/AIDS control activities and other infectious diseases, not only for Kano and other neighbouring States but also for Niger and Cameroon Republics.

Study design and period:

This was a cross sectional, hospital-based study of 155 patients with suspected pulmonary TB aged 10 years and above in January and February 2024, to determine the prevalence of pulmonary mycoses and to detect *M. tuberculosis* (by GeneXpert) among them.

Ethical approval, patient consent and questionnaire:

Ethical approval was obtained from the Health Research Ethics Committee of Kano State Ministry of Health (NHREC/17/03/2018). Prior to specimen collection, informed consent form was designed and signed by all recruited patients. A structured questionnaire capturing socio-demographic data (age, sex, etc) and risk factors for pulmonary mycoses (cigarette smoking, HIV etc) was administered to all consented participants.

Sample size determination:

The Fischer formula, $N=Z^2pq/d^2$ was used to determine the sample size of participants, where N=sample size, Z=standard normal distribution at 95% confidence interval (=1.96), p=prevalence of 9.7% (0.097) from the study of Ocansey et al., (7), q=1–p, and d=allowable error of 5% (0.05). This gave a calculated sample size of 134.6, which was adjusted to 155.

Inclusion and exclusion Criteria:

This included both male and female participants 10 years and above who presented with clinical symptoms and signs of PTB (chronic cough, night sweats, weight loss, fever, and history of contact with person with chronic cough etc) irrespective of HIV status, and consented to participate in the study. Patients with chronic conditions such as diabetes mellitus, chronic obstructive pulmonary diseases, and patients with a history of smoking or alcohol use, were also included.

Patients with extrapulmonary complications or miliary TB, those on antifungal drugs and those who did not consent to participate in the study, were excluded.

Sample collection:

About 4 ml of early morning sputum sample was collected from each study participant into a sterile, wide-neck, transparent plastic leak-proof container in line with NTBLCP standard operating procedures (8), and transported to the medical microbiology unit of the laboratory department of IDH for processing on the same day of collection. Samples not processed on the same day were stored in the refrigerator at 4–8°C until processed.

Detection of *M. tuberculosis* in sputum sample by GeneXpert assay:

Mycobacterium tuberculosis from the sputum samples was detected using the GeneXpert assay machine (Cepheid, Sunnyvale, CA, USA) according to the manufacturer's instructions. Briefly, the sputum sample was mixed with sample reagent in a ratio of 2:1 and allowed to settle at room temperature. Subsequently, 2 ml of the mixture was transferred into the GeneXpert cartridge and loaded into the GeneXpert machine for 2 hours. The results were read and recorded as MTB not detected, MTB detected, RIF resistance not detected, or RIF resistance detected (9).

Isolation and identification of pulmonary fungal pathogens (PFP):

All specimens were analysed by direct microscopy using KOH 10% mount, Gram stain and culture for isolation and detection of fungi. Fungal identification was done by microscopic examination of lactophenol cotton blue (LPCB) stain slides and by germ tube test.

Potassium hydroxide (KOH) 10% wet mounts:

With the aid of Pasteur pipette, a large drop of KOH 10% was placed on the centre of a clean glass slide. A small portion of the purulent part of sputum sample was transferred into the KOH drop with a sterile wire loop and was mixed well, covered with cover slip and placed in a moist chamber at room temperature for 30 minutes. The preparation was then examined under a compound light microscope using low power (10x and 40x objectives) for the presence of fungal elements (10).

Gram stain for yeast identification:

Briefly, a smear was made from the sputum samples on a clean glass slide. The cells and cell fragments were dried on the slide and stained with standard Gram stain as described in Cheesbrough (10). The presence of pseudo hyphae in the sputum was seen as Gram-positive yeast-like cells.

For differential staining, the slide was treated for 5 min with 1% phosphomolybdic acid, washed, and stained for 0.5 min with 1% aqueous methyl green. Whole or intact yeast cells appeared dark purple or black, while broken yeast cells with exposed cytoplasm were stained light purple.

Fungal culture:

Sputum samples were streaked onto Sabouraud Dextrose Agar (SDA) supplemented with and without antibiotic (gentamicin 40 mg/L) to isolate pure culture. The plates were inverted and incubated at 25–30 °C. The fungal growth on SDA plates was examined after 7 days and held for 4 weeks before being reported as negative. The morphology, texture, growth rate, colony/cottony surface, and pigmentation on the surface of SDA tubes were recorded. The fungal isolates on culture were identified using standard mycological procedures. A routine examination of the culture plates was done to exclude any fungal growth at least 3 times a week (10).

Lactophenol cotton blue (LPCB) stain:

A section of small fragment of fungal growth was transferred on to a drop of ethanol placed on a clean, grease-free glass slides. Few drops of LPCB were then added and the slide covered with cover slip. Excess LPCB stain was removed with blotting paper. The preparation was then placed in a moist chamber and kept at room temperature for 30 minutes. The slide was viewed under the microscope using 10x and 40x objectives. The features and characteristics of fungal pathogens seen under the microscope were compared with established characteristic fungal features using mycology atlases.

Germ tube test:

Candida albicans was identified by the germ tube test. Briefly, yeast colonies from the SDA plates were suspended in human serum in a test tube and incubated at 37°C for 3 hours. After observing a faintly turbid suspension, the suspension was examined under the light microscope using 10x and 40x objectives to detect the presence or absence of germ tubes. The result was positive for *C. albicans* if germ tubes arose from the yeast cell and showed matching walls without any narrowing at their point of origin (10).

Statistical analysis of data:

The OpenEPI (version 3.01) statistical online tool was used for data analysis. Chi square test and Odds ratio at 95% confidence interval were used to determine association between categorical variables. A p value < 0.05 was considered statistically significant.

Results:

Prevalence of pulmonary mycoses in the study population:

Table 1 shows the prevalence of pulmonary mycoses among the study population with respect to socio-demographic characteristics (sex and age group). Of the total 155 study participants, 67 (43.2%) were males and 88 (56.8%) were females. A total of 88 (56.8%) participants' sputum samples were positive for pulmonary fungal pathogen (PFP). Females had a higher PFP prevalence of 59.1% (n=52/88) compared to males with 53.7% (n=36/67), but the PFP prevalence difference was not statistically significant (x^2=0.4452, p=0.5046).

Participants in the age groups 20-29 years and 50-59 years had higher PFP prevalence of 67.6% (n=23/34) and 63.0% (n=17/27) respectively, while age groups 10-19 years and ≥60 years had lower PFP prevalence of 50.0% (n=15/30) and 46.7% (n=7/15), respectively. However, the difference in PFP prevalence with respect to age was not statistically significant (x^2=3.534, p=0.6183).

Frequency distribution of fungi isolates among population with pulmonary mycoses:

Among the participants with pulmonary mycoses, *Aspergillus* spp accounted for the highest frequency (n=76, 86.4%) while *C. neo formans* and *H. capsulatum* had the lowest frequencies, each with 2.3% (n=2). The frequency of *Aspergillus* spp was higher in females (n=47, 90.4%) compared to males (n=29, 80.6%) but the difference was not statistically significant (OR=0.4407, 95% CI=0.1278-1.520, p=0.2176). However, higher frequency of *C. albicans* was recorded among male (n=5, 13.9%) than female (n=3, 5.8%) participants, although the difference was also not statistically significant (OR=2.634, 95% CI=0.5874-11.815, p=0.2637).

Prevalence of co-infection of pulmonary myco ses and PTB in the study population:

Of the sputum samples of 155 participants analyzed, 88 (56.8%) and 31 (20.0%) were positive for pulmonary mycoses (by culture) and PTB (by GeneXpert) respectively. In all, 14 (9.0%) were positive for both pulmonary mycoses and PTB, of which males accounted for 6 (8.9%) and females 8 (9.1%).

Table 1: Prevalence of pulmonary mycoses among patients with presumptive pulmonary tuberculosis with respect to sociodemographic characteristics

Characteristics	Fungi culture		Total	x^2	*p* value
	No positive (%)	No Negative (%)			
Sex					
Male	36 (53.7)	31 (46.3)	67	0.4452	0.5046
Female	52 (59.1)	36 (40.9)	88		
Age group (years)					
10-19	15 (50.0)	15 (50.0)	30	3.534	0.6183
20-29	23 (67.6)	11 (32.4)	34		
30-39	14 (53.8)	12 (46.2)	26		
40-49	12 (52.2)	11 (47.8)	23		
50-59	17 (63.0)	10 (37.0)	27		
>60	7 (46.7)	8 (53.3)	15		
Total	**88 (56.8)**	**67 (43.2)**	**155**		

x^2 = Chi square

Table 2: Frequency distribution of fungi isolates among patients with pulmonary mycoses in the study population

Fungi isolate	Number of patients		Total (%)	OR (95% CI)	P value
	Male	Female			
Aspergillus spp	29 (80.6)	47 (90.4)	76 (86.4)	0.4407 (0.1278 - 1.520)	0.2176
Candida albicans	5 (13.9)	3 (5.8)	8 (9.1)	2.634 (0.5874 - 11.815)	0.2637
Cryptococcus neoformans	1 (2.8)	1 (1.9)	2 (2.3)	1.457 (0.08811 - 24.099	1.0000
Histoplasma capsulatum	1 (2.8)	1 (1.9)	2 (2.3)	1.457 (0.08811 – 24.099)	1.000
Total	**36 (40.9)**	**52 (50.1)**	**88 (100.0)**		

OR = Odd ratio; CI=Confidence interval

Table 3: Prevalence of pulmonary mycoses and pulmonary tuberculosis co-infection among the study population

Gender	No of pulmonary mycoses by culture (%)	No of pulmonary TB by GeneXpert (%)	No of pulmonary mycoses-pulmonary TB co-infection (%)
Male (n=67)	36 (53.7)	16 (23.9)	6 (8.9)
Female (n=88)	52 (59.1)	15 (28.8)	8 (9.1)
Total (n=155)	**88 (56.8)**	**31 (20.0)**	**14 (9.0)**

Discussion:

The respiratory tract is the most common site for developing fungal infection and in most cases, colonization is the first step in the progression to pulmonary fungal infection. In this study, sputum samples collected from a total of 155 patients with suspected pulmonary TB were examined, in which 56.8% were positive for pulmonary mycoses, which is similar but slightly lower than the 73.6% reported by Sani et al., (11) among presumptive TB cases in Gombe, Nigeria. Similarly, an Egyptian study also showed that the prevalence of fungal pneumonia in respiratory intensive care unit was 66.7% (12). The slightly lower prevalence rate obtained, might be due to the smaller sample size of this study (samples were collected for a period of 1 month only) when compared to the other two. In all cases, the prevalence of pulmonary mycoses was high.

The male-to-female ratio of 36/52 among the 88 patients with pulmonary mycoses in this study agrees with 60/90 male-to-female ratio reported by Jahromi and Khaksar (13). Sex-related distribution of fungal infections, according to Hidalgo and Vazquez (14), as cited by Ndukwu et al., (15) are equal. Other studies such as that of Yahaya et al., (16) and

Bansod and Rai (3), have reported higher prevalence among males than females, attributing that males are more vulnerable to fungal infections because they are more exposed to external environment where fungi and their spores are in significant numbers. It could also be due to the higher number of female patients in the overall study population, since females generally have higher hospital attendance than males.

Fungal infections of the respiratory tract by large are considered to be identical with invasive pulmonary infections caused by *Aspergillus* spp (2). Our findings are in consistent with this report because out of 155 mycelial fungi recovered in the present study, *Aspergillus* spp accounted for the highest (n=76, 86.4%) proportion while *C. neoformans* and *H. capsulatum* had the lowest, each with 2.3%. Among species of *Aspergillus*, *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* are pathogenic species to man. Most previous studies reported that *A. fumigatus* is the most common cause of pulmonary mycosis (11,17,18).

Candida albicans was the second-most common fungal pathogen isolated in this study, accounting for 9.1%. This is similar to the study of Sani et al., (11) in which *C. albicans* was also reported to be the second-most prevalent fungal isolate and common in co-infection with pulmonary TB. *Candida albicans* is an opportunistic fungal pathogen that affects individuals with a prior history of pathogenic infectious disease with prolonged treatment therapy (19). The high occurrence of this species among pulmonary TB patients indicates fungal colonization due to the extended use of antibiotics. *Candida albicans* was previously reported and has remained the most frequently isolated *Candida* species in the clinical setting (20).

While the prevalence of 56.8% of pulmonary mycosis in presumptive PTB patients in this study is quite similar to the prevalence of 50.0% for chronic pulmonary aspergillosis in patients with presumptive pulmonary TB in Ghana (7), the prevalence of pulmonary mycoses in confirmed pulmonary TB patients is considerably lower. For example, the prevalence of pulmonary fungal pathogen in patients with confirmed pulmonary TB at the Federal Teaching Hospital, Gombe, Nigeria was 6.0% (11), similar to 9.0% that was obtained in our current study. The similarity in the prevalence of co-infection might be due to the study area and study population, where both locations are in northern part of Nigeria having similar climates and environmental conditions with population of same or similar cultural practices. As emphasized by Sule et al., (21), the prevalence of pulmonary mycoses among patients with confirmed pulmonary TB (co-infection) progressively increases, majorly, as a result of anti-TB drugs with non-specific action and depressed immunity, which enable fungi to proliferate.

Conclusion:

From the findings of this study, TB patients are in many cases co-infected with opportunistic pulmonary mycotic agents with *Aspergillus* spp being the most frequent fungal pathogen but also by *C. albicans, C. neoformans* and *H. capsulatum.* There is need for routine screening of all patients with suspected pulmonary TB for pulmonary mycoses, especially in high TB-burden regions, as this will contribute to improved management, and reduction in mortality and morbidity.

Acknowledgements:

The authors appreciate the efforts and assistance rendered by Nazir Lawan Habib (MLS) and staff of the medical microbiology unit, Infectious Disease Hospital, Kano, during the course of the study.

Contributions of authors:

Both authors contributed to conceptualization and design of the study, data analysis and interpretation of results. NMI performed laboratory analysis and ABS drafted the manuscript.

Source of funding:

Authors received no external funding.

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Bitew, A., and Bati, S. Profiling of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens in presumptive tuberculosis patients referred to Saint Peter's Specialized Tuberculosis Referral Hospital, Addis Ababa, Ethiopia. Sage Open Med. 2021; 9: 20503121211056163. doi:10.1177/20503121211056163.
2. Chowdhary, A., Agarwal, K., and Meis, J. F. Filamentous fungi in respiratory infections. What lies beyond aspergillosis and mucormycosis? PLoS Pathog. 2016; 12: e1005491. doi: 10.1371/journal.ppat.1005491.

3. Bansod, S., and Rai, M. Emerging of mycotic infection in patients infected with *Mycobacterium tuberculosis*. World J Med Sci. 2008; 3: 74-80.
4. Mathavi, S., Shankar, R., Sasikala, G., and Kavitha, A. A Study on mycotic infections among sputum positive pulmonary tuberculosis patients in Salem district. Paripex-Indian J Res. 2015; 4: 299-302.
5. Muni, S., Rajpal, K., Kumar, R., et al. Identification of fungal isolates in patients with pulmonary tuberculosis treated at a Tertiary Care hospital. Cureus. 2023; 15 (4): e37664. doi: 10.7759/cureus.37664.
6. Amiri, M. R. J., Siami, R., and Khaledi, A. Tuberculosis status and coinfection of pulmonary fungal infections in patients referred to reference laboratory of Health Centers Ghaemshahr City during 2007-2017. Ethiop J Health Sci. 2018; 28: 683-690. doi: 10.4314/ejhs.v28i6.2.
7. Ocansey, B. K., Otoo, B., Adjei, A., et al. Chronic pulmonary aspergillosis is common among patients with presumed tuberculosis relapse in Ghana. Med Mycol. 2022; 60 (9): myac063. doi:10.1093/mmy/myac063.
8. National Tuberculosis, Leprosy and Buruli Ulcer Control Programme Guidelines (NTBLCP). Standard Operating Procedure for the Detection of MTB complex and Resistance to Rifampicin from extrapulmonary samples using Xpert MTB/RIF assay in Nigeria. Federal Ministry of Health, Department of Public Health. 2017.
9. Centers for Disease Control and Prevention (CDC). A new tool to diagnose tuberculosis: The Xpert MTB/RIF Assay. 2015. https://findtbresources.cdc.gov/view?id=323173.
10. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 1. Second Edition. Cambridge University Press, UK. 2002.
11. Sani, F. M., Uba, A., and Tahir, F. 'Spectrum of Pulmonary Fungal Pathogens, Associated Risk Factors and Antifungal Susceptibility Pattern among Persons with Presumptive Tuberculosis at Gombe, Nigeria. Int J Mycobacteriol. 2020; 9 (2): doi: 10.4103/ijmy.ijmy_46_20.
12. Ahmed, M., Farghaly, A., Raafata, R., and AbdElsattar, W. Study of the prevalence and pattern of fungal pneumonia in respiratory intensive care units. Egypt J Bronchol. 2019; 13 (4): 545-550. doi:10.4103/ejb.ejb_37_19
13. Jahromi, and Khaksar, A. A. Study of systemic fungal infection. Hakin Res J. 2002; 4(4): 229-237
14. Hidalgo, J. A., and Vazquez, J. A. Candidiasis. E-medicine. 2012. http://emedicne.medscape.com/article/213853-overview.
15. Ndukwu, C. B., Mbakwem-Aniebo, C., and Frank-Peterside, N. Prevalence of *Candida* co-infection among patients with pulmonary tuberculosis in Emuoha, Rivers State, Nigeria. IOSR-JPBS. 2016; 11 (5): 60-63. doi: 10.9790/3008-1105036063.
16. Yahaya, H., Taura, D. W., Aliyu, I. A., et al. Spectrum of opportunistic mould infections in suspected pulmonary tuberculosis (TB) patients. Int J Microbiol Appl. 2015; 2 (1): 6-11.
17. Lucena, P., Barberán, J., and Eroles, G. Significance of lower respiratory tract cultures yielding Aspergillus spp. growth in a hospital without transplant patients. Rev Esp Quimioter. 2010; 23: 190–195.
18. Mwaura, N. E., Elizabeth, V. M., and Christine, B. Mycological findings of a sputum sample from PTB patients attending TB clinic Nairobi, Kenya. Virol Mycol. 2013; 2: 119. doi; 10.4172/2161-0517.1000119
19. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland: World Health Organization, 2020. https://www.who.int/publications/i/item/9789240013131.
20. Parkes-Ratanshi, R., Achan, B., and Kwizera, R. Cryptococcal disease and the burden of other fungal diseases in Uganda; where are the knowledge gaps and how can we fill them? Mycoses. 2015; 58: 85–93. doi: 10.1111/myc.12387.
21. Sule, H., Kumurya, A. S., and Adamu, A. I. Mycosis as co-infection among Tuberculosis Infected patients in some countries: A Review. Dutse Journal of Pure and Applied Sciences (DUJOPAS). 2019; 5 (2a): 237-244.

Maktep et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 385 - 390 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27; No.3
AJCEM/2506. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Relationship between serum viral load and stages of hepatic fibrosis in patients with chronic hepatitis C virus infections in Plateau State, Nigeria

[1,2,3]Maktep, Y, D., [1,2]Egah, D. Z., and [1]Nimzing, L.

[1]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Health Sciences, University of Jos, PMB 2084 Jos, Nigeria
[2]Department of Medical Microbiology, Jos University Teaching Hospital, Jos, Nigeria
[3]Department of Medical Microbiology and Parasitology, College of Health Sciences, Bingham University, Jos Campus, Nigeria
*Correspondence to: yadangmaktep@gmail.com

Abstract

Background: Chronic hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide and remains a significant contributor to liver-related morbidity and mortality. Hepatic fibrosis is the key determinant of long-term clinical outcomes. Although viral replication is central to HCV pathogenesis, the association between serum HCV load and the severity of hepatic fibrosis remain controversial. The objective of this study is to evaluate the relationship between serum HCV load and hepatic fibrosis severity, using Transient Elastography (FibroScan), among patients with chronic HCV infection.
Methodology: A descriptive cross-sectional study with longitudinal follow up of confirmed cases was conducted among 40 adults with confirmed chronic HCV infection (HCV-RNA positive). Serum viral load was quantified using real-time reverse-transcription polymerase chain reaction (rt RT-PCR) assay. Hepatic fibrosis was assessed non-invasively using FibroScan, and liver stiffness measurements (LSM) were categorized according to METAVIR-equivalent cut-offs of F0 (<5.5 kPa), F1 (5.5–7.0 kPa), F2 (7.1–9.5 kPa), F3 (9.6–12.5 kPa), and F4 (>12.5 kPa). Spearman's correlation coefficient was used to determine the association between viral load and fibrosis severity as the data deviated significantly from the normality.
Results: The mean age of participants was 56.10±15.73 years with a male-to-female ratio of 4:1. Liver stiffness values ranged from 4.7 to 35.8 kPa, while serum viral load varied widely (20,100 to 680,000 IU/mL). Most participants had early-stage fibrosis (F0–F1, 70%). A weak positive correlation was observed between serum viral load and liver stiffness measurements (r=0.139), that was not statistically significant (p=0.391).
Conclusion: Serum HCV load showed no significant association with hepatic fibrosis severity. These findings indicate that viral load alone is a poor predictor of fibrosis progression and underscore the clinical value of Transient Elastography for routine assessment of liver disease in patients with chronic HCV infection.

Keywords: HCV; viral load; hepatic fibrosis; FibroScan

Received Mar 7, 2026; Revised Apr 27, 2026; Accepted Apr 28, 2026

Relation entre la charge virale sérique et les stades de fibrose hépatique chez les patients atteints d'une infection chronique par le virus de l'hépatite C dans l'État de Plateau, au Nigéria

[1,2,3]Maktep, Y. D., [1,2]Egah, D. Z., et [1]Nimzing, L.

[1]Département de Microbiologie Médicale, Faculté des Sciences Cliniques, Collège des Sciences de la Santé, Université de Jos, PMB 2084 Jos, Nigéria
[2]Département de Microbiologie Médicale, Centre Hospitalier Universitaire de Jos, Jos, Nigéria
[3]Département de Microbiologie Médicale et de Parasitologie, Collège des Sciences de la Santé, Université Bingham,

Campus de Jos, Nigéria
*Correspondance à: yadangmaktep@gmail.com

Résumé:

Contexte: L'infection chronique par le virus de l'hépatite C (VHC) est une cause majeure de maladie hépatique chronique dans le monde et contribue de manière significative à la morbidité et à la mortalité liées au foie. La fibrose hépatique est le principal déterminant des résultats cliniques à long terme. Bien que la réplication virale soit essentielle à la pathogenèse du VHC, l'association entre la charge virale sérique du VHC et la sévérité de la fibrose hépatique reste controversée. L'objectif de cette étude est d'évaluer la relation entre la charge virale sérique du VHC et la sévérité de la fibrose hépatique, par élastographie impulsionnelle (FibroScan), chez des patients atteints d'une infection chronique par le VHC.
Méthodologie: Une étude descriptive transversale avec suivi longitudinal des cas confirmés a été menée auprès de 40 adultes atteints d'une infection chronique confirmée par le VHC (ARN du VHC positif). La charge virale sérique a été quantifiée par RT-PCR en temps réel. La fibrose hépatique a été évaluée de manière non invasive par FibroScan, et les mesures de rigidité hépatique (MRH) ont été classées selon les seuils équivalents à ceux de METAVIR: F0 (< 5,5 kPa), F1 (5,5–7,0 kPa), F2 (7,1–9,5 kPa), F3 (9,6–12,5 kPa) et F4 (> 12,5 kPa). Le coefficient de corrélation de Spearman a été utilisé pour déterminer l'association entre la charge virale et la gravité de la fibrose, car les données s'écartaient significativement de la normalité.
Résultats: L'âge moyen des participants était de 56,10±15,73 ans, avec un ratio hommes/femmes de 4/1. Les valeurs de rigidité hépatique variaient de 4,7 à 35,8 kPa, tandis que la charge virale sérique présentait une grande variabilité (de 20 100 à 680 000 UI/mL). La plupart des participants présentaient une fibrose à un stade précoce (F0–F1, 70%). Une faible corrélation positive a été observée entre la charge virale sérique et les mesures de rigidité hépatique (r=0,139), mais cette corrélation n'était pas statistiquement significative (p=0,391).
Conclusion: La charge virale sérique du VHC n'a montré aucune association significative avec la sévérité de la fibrose hépatique. Ces résultats indiquent que la charge virale seule est un mauvais prédicteur de la progression de la fibrose et soulignent l'intérêt clinique de l'élastographie impulsionnelle pour l'évaluation de routine des maladies hépatiques chez les patients atteints d'une infection chronique par le VHC.

Mots-clés: VHC; charge virale; fibrose hépatique; FibroScan

Introduction:

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease worldwide, which may progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma if untreated (1,2, 3,4). The extent of hepatic fibrosis is a critical determinant of prognosis, therapeutic decision-making, and long-term patient monitoring (2, 5). Historically, liver biopsy served as the 'gold standard' for fibrosis assessment, however, its invasive nature, sampling variability and potential complications have limited its routine use (6,7). Consequently, non-invasive methods such as Transient Elastography (FibroScan), have gained widespread acceptance due to their safety, reproducibility, and strong correlation with histological fibrosis stages (5,8,9). Also, liver stiffness can be quantified as a continuous variable and can help physicians in grading the severity of hepatic fibrosis in order to estimate the likelihood of developing liver related complications including hepatocellular carcinoma (10,11,12).

The clinical relevance of quantitative serum HCV-RNA levels in predicting severity of hepatic fibrosis remains uncertain (4). While viral replication is essential for disease initiation and persistence, studies have reported inconsistent associations between viral load and fibrosis progression (13,14). There is increasing evidence to suggest that host-related factors, including immune response, duration of infection, metabolic comorbidities, alcohol consumption, and age, may exert a greater influence on fibrogenesis than circulating viral RNA levels alone (13,15).

In resource-limited settings, clinicians often rely on readily available laboratory parameters such as viral load for risk stratification. Clarifying the relationship between serum viral load and hepatic fibrosis is therefore of practical clinical importance. This study aimed to evaluate the association of serum HCV load on hepatic fibrosis severity using FibroScan as a non-invasive assessment tool.

Materials and method:

Study design and setting:

This was a descriptive cross-sectional study of adult patients (18 years and above) with suspected hepatitis, and a longitudinal follow up of confirmed cases, in 9 selected Primary Healthcare Centers (PHCs) within the 3 senatorial districts of Plateau State, Nigeria.

Ethical consideration:

Ethical approval was obtained from the Health Research Ethics Committee of Plateau State Specialist Hospital (NHREC/09/23/2010b),

Jos, Nigeria. Written informed consent was obtained from all participants prior to enrollment, and the study was conducted in accordance with the Declaration of Helsinki

Study population and sample size

The study population were adult patients (18 years and above) with suspected hepatitis attending the general outpatient departments (GOPD) of the selected PHCs. The minimum sample size was calculated using the formula described by Araoye (16), $n=Z^2pq/d^2$ where N=minimum sample size, Z= standard normal deviation corresponding to 95% levels of significance (=1.96), p=prevalence rate of HCV of 18.4% (0.184) in the study by Uchendu et al., (17), q=1-p and d is the degree of accuracy desired, set at 5% (0.05). This gave a calculated sample size of 231, which was adjusted for 15% attrition to 266, but a total of 461 participants were recruited to increase the power of the study.

Selection criteria and method of sampling:

The inclusion criteria included patients aged 18 years and above who consented to participate in the study and without prior knowledge of their HCV status. Patients less than 18 years of age and those with history of confirmed HCV infection were excluded.

The local government areas and PHCs within each of the senatorial zones were selected by simple random sampling. The patients presenting to GOPD of the PHCs who consented to participate in the study were recruited by simple random sampling technique until the sample size was reached.

Data and sample collection:

Data on socio-demographic characteristics from each participant were collected using a structured, interviewer-administered questionnaire. Five milliliters of venous blood were collected aseptically from each participant into EDTA vacutainer. Plasma was separated by centrifugation at 2000 rpm for 5 minutes and stored at −20°C until laboratory analysis.

Preliminary screening for HCV by ELISA and confirmation by PCR:

Preliminary screening of all 461 participants for HCV antibodies was done by the Enzyme Linked Immunosorbent Assay (ELISA kit, DIAPRO Diagnostics, Italy). Forty-nine participants positive for HCV antibodies were confirmed HCV-RNA positive using HCV-RNA PCR (Luna PCR kit, New England BioLabs, Germany), and after a minimum follow-up period of six months, 40 participants were successfully enrolled for quantitative viral load analysis and fibrosis assessment.

Quantitative HCV load testing:

HCV-RNA quantification was performed using the Bosphore® HCV Quantification Kit on the Bio-Rad CFX96 Real-Time PCR system in accordance with the manufacturer's instructions. Viral RNA extraction was carried out using the Quick Viral RNA Extraction Spin Kit. Results were expressed in IU/mL.

Hepatic fibrosis assessment:

Hepatic fibrosis was assessed using Transient Elastography (FibroScan). Liver stiffness measurements were recorded in kilopascals (kPa) and categorized according to METAVIR-equivalent cut-offs: F0 (<5.5 kPa), F1 (5.5–7.0 kPa), F2 (7.1–9.5 kPa), F3 (9.6–12.5 kPa), and F4 (>12.5 kPa) (18).

Statistical analysis:

Data were analyzed using descriptive statistics. Normality test was assessed using Kolmogorov-Smirnov and Shapiro–Wilk tests. Spearman's rank correlation coefficient was applied to evaluate the relationship between serum viral load and liver stiffness measurements since the variables were not normally distributed. Statistical significance was set at $p<0.05$.

Results:

Socio-demographic and clinical characteristics of the study participants:

Table 1 shows the sociodemographic and clinical characteristics of the 40 enrolled study participants. The mean age of the participants was 56.10±15.73 years, with 80% males and 20% females. Majority of the participants were in the age groups 40–49 years and 50-59 years, accounting for 40% each of the study population.

The liver fibrosis assessment using FibroScan showed that the highest frequency of participants had stage F_1 fibrosis (40.0%), followed by stage F_0 (30.0%), while the lowest frequencies of the participants were classified as F_2, F_3 and F_4, accounting for 10% each. The FibroScan scores ranged from 4.7 to 38.8 Kpa (Kilopascal). The HCV load ranged from 20,100 to 680,000 IU/mL.

Table 1: Socio-demographic and clinical characteristics of the study participants

Variables	Frequency	Percentage
Age group (years)		
40-49	16	40.0
50-59	16	40.0
≥60	8	20.0
Mean age (± SD)	56.10 ± 15.73	
Gender		
Male	32	80.0
Female	8	20.0
FibroScan stage		
F_0	12	30.0
F_1	16	40.0
F_2	4	10.0
F_3	4	10.0
F_4	4	10.0

Table 2 shows association between age, gender and hepatic fibrosis among the participants. There was a statistically significant association between age and stages of hepatic fibrosis (x^2=34.164, p=0.001) with early-stage fibrosis (F0, F1) being significantly commoner in middle age (40-49 years age group) while late-stage fibrosis (F2, F3, F4) was commoner in older adults (>50 years of age). However, there was no significant association between gender and stages of hepatic fibrosis (x^2=4.58, p=0.333).

Table 3 shows the assessment result of the normality distribution of the variables (FibroScan scores and HCV load) using Kolmogo rov–Smirnov and Shapiro wilk tests. Both FibroScan scores and HCV load significantly deviated from normal distribution (p=0.001 for both), which necessitated the use of Spearman's correlation.

Table 4 shows the Spearman's correlation between HCV load and stages of hepatic fibrosis. A weak positive correlation was observed between HCV load and stages of hepatic fibrosis (r=0.139), which was not statistically significant (p=0.391).

Table 2: Relationship between stages of hepatic fibrosis and demographic characteristics of the participants

Demographic characteristics	Stages of hepatic fibrosis					Total	χ^2	*p* value
	F_0	F_1	F_2	F_3	F_4			
Age group (years)								
40-49	8 (50.0)	8 (50.0)	0	0	0	16	34.167	0.001
50-59	4 (25.0)	4 (25.0)	0	4 (25.0)	4 (25.0)	16		
≥60	0	4 (50.0)	4 (50.0)	0	0	8		
Gender								
Male	8 (25.0)	12 (37.5)	4 (12.5)	4 (12.5)	4 (12.5)	32	4.583	0.333
Female	4 (50.0)	4 (50.0)	0	0	0	8		

Table 3: Tests of normality for HCV load and liver stiffness measurement

Parameter	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	*p* value	Statistic	Df	*p* value
HCV load	0.200	40	0.001	0.864	40	0.001
FibroScan score	0.360	40	0.001	0.478	40	0.001

df = degree of freedom; HCV = Hepatitis C virus

Table 4: Spearman correlation analysis between serum HCV load and hepatic FibroScan score

			HCV load	FibroScan score
Spearman's rho	**HCV load**	Correlation coefficient	1	0.139
		p value		0.391
		Number	40	40
				1
	FibroScan score	Correlation coefficient	0.139	
		p value	0.391	
		Number	40	40

HCV = Hepatitis C virus

Discussion:

The study evaluated the correlation between serum HCV load and hepatic fibrosis severity assessed using transient Elastography (FibroScan) among the participants with confirmed chronic HCV infection. The findings showed that there was no statistically significant association between HCV load and the severity of hepatic fibrosis. Although, a weak positive correlation was observed between HCV load and liver stiffness measurement (FibroScan score), the correlation was not statistically significant, suggesting that serum HCV load alone may not be a reliable predictor for progression of liver fibrosis in chronic HCV infected patients.

The sociodemographic characteristics of the study participants revealed that most were middle-aged aged (40-49 years) and older adults (50-59 years), indicating that prevalence of HCV infections are higher in middle age and older adults in the study population. This finding is consistent with the natural history of chronic HCV infection in which the fibrosis of the liver develops progressively over many years before clinical manifestation occurs (7,19). This finding is similar to previous reports by other researchers (7,20). The predominance of male participants in this study may be related to increased exposure to occupational and behavioral risk factors associated with bloodborne viral infections.

Notably, 10% of the participants already had stage 4 (F4) fibrosis despite having no prior knowledge of their HCV status. This suggests that many individuals in the community in Plateau State remains unaware of their HCV status and may eventually presents with severe complication in healthcare facilities. This finding is in tandem with previous reports by Pandey and co-workers (21) that many individuals in the community are unaware of their HCV status and remain undiagnosed until complications become evident. Forty percent of the participants were found with mild fibrosis (F1) which may be due to early detection or variation in the progression of HCV infection among the participants.

The serum HCV load and liver stiffness measurement values (FibroScan scores) both shows wide variability among the study population reflecting heterogeneity in viral replication activity and the severity of the HCV infection. A statistically significant association was observed between age and stages of fibrosis, with higher frequency of advanced fibrosis observed among older age group (50-59 years) compared to younger age group (40-49 years). This finding agrees with previous reports by Zeremski et al., (2) and Arshad & coworkers (7), and may be attributed to prolong duration of infection, cumulative hepatic injury over time and/or decline in hepatic regenerative activity with advancing age. The association between gender and stages of hepatic fibrosis was not statistically significant although males were more represented across the fibrosis stages compared to female participants. The lack of significance may be due to relatively small sample size.

The normality tests revealed that both FibroScan scores and HCV load were not normally distributed. The significant deviation from normality could be attributed to the wide variation in HCV load and FibroScan scores seen among the participants. By Spearman's rank correlation analysis, there was weak correlation coefficient (r=0.139) between HCV load and severity of liver fibrosis, which was not statistically significant (p=0.391). This implies that increasing HCV load does not necessarily correspond to worsening of liver fibrosis. HCV load reflects viral replication activities and not necessarily the degree of hepatic structural damage. This finding suggests that hepatic injury and fibrogenesis in chronic HCV infection may be influenced by host immune responses and other clinical or metabolic factors than by circulating RNA level alone (13,14). The predominance of early-stage fibrosis observed in this study despite markedly elevated viral load in some patients further strengthens this observation.

These findings agree with those of other studies that reported no significant correlation between serum HCV load and severity of hepatic fibrosis (13,14). However, some studies have reported contrasting findings of significant correlation between serum HCV load and hepatic fibrosis severity (4,7). The discrepancies across studies may be explained by differences in study design, population characteristics, viral genotypes, duration of infection and environmental or genetic factors such as obesity, alcohol use, viral coinfection and insulin resistance.

The finding of this study has important clinical implications especially in resource-limited settings where HCV load determination may often be interpreted as indicator of HCV disease severity. While the HCV load remains an important aspect in confirming active infection and monitoring treatment, this study suggest that it should not be used independently to predict severity of hepatic fibrosis. Assessing hepatic fibrosis using FibroScan remains an important tool for the evaluation of hepatic fibrosis and guiding clinical management in

patients with chronic HCV infection.

This study is limited by relatively small sample size, which limits the generalizability of the findings. The cross-sectional design also precludes causal inference between viral load and fibrosis progression. Additionally, liver biopsy, the histological 'gold standard' was not performed; however, the FibroScan used is a validated and widely accepted non-invasive alternative. Furthermore, information on viral genotype, duration of infection, alcohol consumption, and metabolic comorbidities was limited and may have influenced fibrosis severity.

Conclusion:

In this study, serum HCV load was not significantly associated with hepatic fibrosis severity and should therefore not be used in isolation to predict fibrosis progression. Transient Elastography (FibroScan) remains a valuable non-invasive tool for fibrosis assessment, particularly in resource-limited settings. Future studies incorporating intrahepatic viral replication and immunologic markers are recommended to further elucidate determinants of fibrosis progression in chronic HCV infection.

Contributions of authors:

MY conceived and designed the study, coordinated data collection and laboratory analyses, performed FibroScan assessments, and contributed to data interpretation. EZD and NL provided supervision and critical intellectual input. All authors contributed to manuscript drafting, reviewed the final version, and approved the manuscript for submission.

Source of funding:

This study received no funding.

Conflicts of interest:

Authors declare no conflicts of interest.

References

1. Leone, N., and Rizzetto, M. Natural history of hepatitis C infection: from chronic hepatitis to cirrhosis and hepatocellular carcinoma. Minerva Gastroenterol Dietol. 2005;51 (1);31-46.
2. Zeremski, M., Dimova, R. B., Pillardy, J., de Jong, P., Jacobson, J. M., and Talal, A. H. Fibrosis progression in patients with chronic hepatitis C virus infection. J Infect Dis. 2016; 214 (8): 1164-1170. doi:10.1093/infdis/jiw332.
3. Malone, C. D., Fetzer, D. T., Monsky, W. L, et al. Contrast-enhanced ultrasound for interventional radiologists: current and emerging applications. Radiographics. 2020; 40 (2): 562-588. doi:10.1148/rg.2020190183.ed
4. Faheem, M., Iqbal, N., Hussain, T., and Qureshi, A. Association between quantitative viral load and severity of liver fibrosis in chronic viral hepatitis patients: a cross-sectional study. J Health Well- being Clin Res. 2025; 4 (1): 45-53.
5. Lai, J. C., Yang, L. L., and Wong, G. L. H. Non-invasive tests for liver fibrosis in 2024: are there different scales for different diseases? Gastro- enterol Rep (Oxf). 2024;12: goae024. doi:10.1093/gastro/goae024.
6. Regev, A., Berho, M., Jeffers, L. J., et al. Sampling error and intra-observer variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002; 97 (10): 2614-2618. doi:10.1111/j.1572-0241.2002.06038.x.
7. Arshad, M., Manzoor, I., Memon, K. H., et al. Association of fibrotic changes in the liver on FibroScan with viral load and duration of hepatitis C infection: a pilot study. J Ayub Med Coll Abbottabad. 2023; 35 (1): 73-78.
8. Bacon, B. R. Treatment of patients with hepatitis C and normal serum aminotransferase levels. Hepatol. 2002; 36 (5 Suppl 1): S179-S184. doi:10.1053/jhep.2002.36586.
9. Foucher, J., Chanteloup, E., Vergniol, J., et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut. 2006; 55 (3): 403-408. doi:10.1136/gut.2005.069153.
10. Kazemi, F., Kettaneh, A., N'kontchou, G., et al. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. J Hepatol. 2006;45(2):230-235. doi:10.1016/j.jhep.2006.04.006.
11. Kim, B. K., Han, K. H., Park, J. Y., et al. A liver stiffness-based non-invasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. Am J Gastroenterol. 2010; 105 (6): 1382-1390. doi:10.1038/ajg.2009.750.
12. Jung, K. S., Kim, S. U., Ahn, S. H., et al. Risk assessment of hepatitis B-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatol. 2011; 53 (3): 885-894. doi:10.1002/hep.24121.
13. Anand, B. S., and Velez, M. Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease. World J Gastroenterol. 2004; 10 (16): 2409-2411. doi:10.3748/wjg.v10.i16.2409.
14. Xu, Z., Lin, J., Lin, G., et al. Hepatitis C virus load in parenchymal cells correlates with hepatic injury in infected patients. Exp Ther Med. 2017; 13 (1): 155-159. doi:10.3892/etm.2016.3915.
15. Cerny, A., and Chisari, F. V. Pathogenesis of chronic hepatitis C: immunological features of hepatic injury and viral persistence. Hepatol. 1999;30 (3): 595-601. doi:10.1002/hep.510300320.
16. Araoye, M. O. Sample Size Determination in Research Methodology with Statistics for Health and Social Sciences. Nathadex Publishers, Ilorin, 2004.
17. Uchendu, T, A., Daboer, J. T., and Amaike, C. The burden of hepatitis C virus infection and access to treatment among rural dwellers in a North Central Nigeria. Jos J Med. 2019; 13 (2): 68-70
18. Castera, L., Forns, X., and Alberti, A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008; 48: 835–847. doi:10.1016/j.jhep.2008.02.008
19. Dhawan, V. K., and Anand, B. S. Hepatitis C. Medscape. Updated 2024. https://emedicine.medscape.com/article/177792-overview
20. Yosry, A., Fouad, R., Alem, S. A., et al. FibroScan, APRI, FIB-4 and GUCI: role in prediction of fibrosis and response to therapy in Egyptian patients with HCV infection. Arab J Gastroenterol. 2016; 17 (2): 78-83. doi:10.1016/j.ajg.2016.04.001.
21. Pandey, G., Gyawali, M., Dhungana, N. S., et al. Distribution of hepatitis C genotypes and viral load pattern among hepatitis C positive patients of different age groups in Nepal. J Hepatol Gastrointestinal Dis. 2022; 8: 212. doi:10.4172/2475-3181.1000212

Pishva. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 391 - 394 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2501. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Integron-mediated multidrug resistance in uropathogenic *Escherichia coli* isolates from intensive care unit: Molecular evidence from Iran within a Global Surveillance Framework

Ebtehaj Pishva

Independent Researcher (Allied Health Assistance), Sydney, Australia
Correspondence to: ebtehajpishva2015@gmail.com

Abstract:

Background: Integrons are genetic platforms that facilitate acquisition and expression of antimicrobial resistance gene cassettes. Their contribution to multidrug resistance (MDR) among intensive care unit (ICU) uropathogenic *Escherichia coli* (UPEC) is of increasing concern. This study determined the prevalence of class 1 and class 2 integrons among ICU UPEC isolates and examined their association with resistance phenotypes.
Methodology: A total of 100 UPEC isolated from ICU patients were identified using standard microbiological techniques. Antimicrobial susceptibility testing against ampicillin, ciprofloxacin and trimethoprim-sulfamethoxazole was performed according to CLSI guidelines. MDR was defined as resistance to the 3 antimicrobial classes. Polymerase chain reaction (PCR) assays targeted *intI1* and *intI2* genes. Association of MDR with integron carriage was analyzed using Chi-square test with $p<0.05$ considered significant.
Results: Resistance rates were highest for ampicillin (40.0%, n=40) and trimethoprim-sulfamethoxazole (40.0%, n=40), while ciprofloxacin resistance was 32.0%. MDR was observed in 56.0% of the isolates. Class 1 integrons were detected in 62.0% and class 2 integrons in 18.0% of the isolates. Class 1 integron carriage was significantly associated with MDR phenotype ($p<0.05$).
Conclusion: These findings underscore the importance of molecular surveillance in ICU settings to guide infection control and antimicrobial stewardship.

Keywords: Integrons; multidrug resistance; uropathogenic *Escherichia coli*; ICU

Received Feb 23, 2026; Revised Apr 8, 2026; Accepted Apr 9, 2026

Résistance aux médicaments multiples par les intégrons chez des isolats d'*Escherichia coli* uropathogènes provenant d'unités de soins intensifs: données moléculaires iraniennes dans le cadre d'une surveillance mondiale

Ebtehaj Pishva

Chercheuse indépendante (Assistance Paramédicale), Sydney, Australie
Correspondance à: ebtehajpishva2015@gmail.com

Résumé:

Contexte: Les intégrons sont des plateformes génétiques qui facilitent l'acquisition et l'expression de cassettes de gènes de résistance aux antimicrobiens. Leur contribution à la multirésistance (MDR) chez *Escherichia coli* uropathogène (UPEC) en unités de soins intensifs (USI) est une préoccupation croissante. Cette étude a déterminé la prévalence des intégrons de classe 1 et de classe 2 parmi les isolats d'UPEC en USI et a examiné leur association avec les phénotypes de résistance.
Méthodologie: Au total, 100 isolats d'UPEC provenant de patients en USI ont été identifiés à l'aide de techniques microbiologiques standard. Des tests de sensibilité aux antimicrobiens (ampicilline, ciprofloxacine et triméthoprime-sulfaméthoxazole) ont été réalisés conformément aux recommandations du CLSI. La multirésistance (MDR) a été

définie comme une résistance aux trois classes d'antimicrobiens. Les tests de réaction en chaîne par polymérase (PCR) ont ciblé les gènes *intI1* et *intI2*. L'association entre la MDR et le portage d'intégrons a été analysée par le test du χ^2 (p<0,05 étant considéré comme significatif).
Résultats: Les taux de résistance les plus élevés ont été observés pour l'ampicilline (40,0%, n=40) et le triméthoprime-sulfaméthoxazole (40,0%, n=40), tandis que la résistance à la ciprofloxacine était de 32,0%. Une MDR a été observée chez 56,0% des isolats. Des intégrons de classe 1 ont été détectés chez 62,0% des isolats et des intégrons de classe 2 chez 18,0% d'entre eux. Le portage d'intégrons de classe 1 était significativement associé au phénotype MDR (p<0,05).
Conclusion: Ces résultats soulignent l'importance de la surveillance moléculaire en soins intensifs pour orienter la lutte contre les infections et l'utilisation judicieuse des antibiotiques.

Mots-clés: Intégrines; multirésistance; *Escherichia coli* uropathogène; soins intensifs

Introduction:

Urinary tract infections (UTIs) are among the most common healthcare-associated infections, particularly in intensive care units (ICUs). Uropathogenic *Escherichia coli* (UPEC) remains the leading causative agent. The global rise of antimicrobial resistance represents a major public health concern (1-3).

Integrons, particularly class 1 integrons, play a crucial role in capturing and disseminating resistance gene cassettes, contributing significantly to the emergence of multidrug-resistant organisms (4). The objective of this study is to determine the prevalence of class 1 and class 2 integrons among ICU UPEC isolates and examined their association with resistance phenotypes.

Materials and method:

Study setting and design:

This laboratory-based study was conducted at Alzahra Hospital, Isfahan, Iran, between January and June 2023.

Clinical samples, bacterial isolation and identification:

A total of 100 non-duplicate ICU isolates were collected after urine samples were cultured on MacConkey and CLED agar, and isolates were identified using standard biochemical tests.

Antimicrobial susceptibility testing:

The Kirby–Bauer disk diffusion method was performed on each isolate according to the 2022 Clinical and Laboratory Standards Institute (CLSI) guidelines (5). The antibiotics tested included ampicillin, ciprofloxacin, and trimethoprim–sulfamethoxazole.

Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes.

Detection of integrons by PCR assay:

DNA was extracted from each isolate using the boiling method. The integrase genes *intI1* (280 bp) and *intI2* (233 bp) were amplified by conventional PCR using *IntI1* (F 5′-CCTCCC GCACGATGATC-3′ and R 5-TCCACGCATCGTCA GGC-3′) and *IntI2* (F 5′-TTATTGCTGGGATTAG GC-3′ and R 5′-ACGGCTACCCTCTGTTATC-3′) primers (6).

The PCR conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. Amplified products were analyzed by agarose gel electrophoresis.

Statistical analysis:

Data were analyzed on SPSS and association of MDR with integron carriage was determined using Chi-square test, with p<0.05 considered statistically significant.

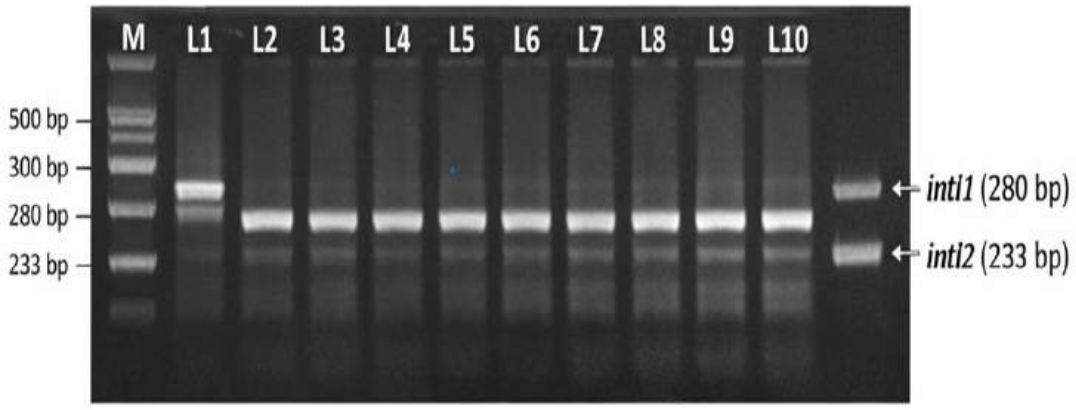
Results:

Of the 100 UPEC isolates, 40 (40.0%) were resistant to ampicillin, 40 (40.0%) to trimethoprim–sulfamethoxazole and 32 (32.0%) to ciprofloxacin. A total of 56 (56.0%) were multidrug resistant.

Class 1 integrons were identified in 62 (62.0%) isolates, while class 2 integrons were detected in 18 (18.0%) isolates. The prevalence of class 1 integrons was significantly associated with MDR isolates compared to non-MDR isolates (p<0.05).

Table 1: Antibiotic resistance among uropathogenic *Escherichia coli* isolates from ICU patients

Antibiotic	No of isolates	No resistant (%)
Ampicillin	100	40 (40.0)
Trimethoprim–sulfamethoxazole	100	40 (40.0)
Ciprofloxacin	100	32 (32.0)
All three antibiotics (MDR)	100	56 (56.0)



Lane M: DNA ladder; Lanes 1–10: ICU UPEC isolates

Fig 1: Agarose gel electrophoresis showing amplification of integron genes *intI1* (280 bp) and *intI2* (233 bp).

Discussion:

The present study demonstrates a high prevalence of class 1 integrons (62%) among ICU-associated UPEC isolates, highlighting their critical role in the acquisition and dissemination of antimicrobial resistance. Integrons function as efficient genetic platforms capable of capturing, integrating, and expressing gene cassettes, thereby facilitating rapid bacterial adaptation under antibiotic selective pressure (7,8, 9). The significant association observed between class 1 integrons and multidrug resistance (MDR) ($p<0.05$) further supports their contribution to the emergence of resistant phenotypes in clinical settings.

The MDR rate of 56% identified in this study is consistent with recent global reports indicating a rising burden of antimicrobial resistance among Enterobacteriaceae. This trend is driven by a combination of horizontal gene transfer and chromosomal mutations, often mediated by mobile genetic elements such as plasmids, transposons, and integrons (10,11,12). The ICU settings represent high-risk environments due to prolonged hospitalization, extensive antibiotic use, invasive procedures, and immunocompromised patients, all of which promote the selection of resistant strains.

Global surveillance reports indicate that integron-mediated resistance is widely distributed across healthcare settings worldwide (13, 14), highlighting the importance of molecular monitoring. The lower prevalence of class 2 integrons (18%) suggests a secondary but still relevant role in resistance dissemination. This finding reinforces the clinical significance of integron screening as part of routine antimicrobial resistance surveillance in high-risk hospital environments.

This study was limited to a single hospital and a defined sample size. Additionally, sequencing of integron gene cassettes was not performed. Future studies should include gene cassette sequencing and multi-centre surveillance to better understand resistance dissemination.

Conclusion:

Class 1 integrons are highly prevalent and significantly associated with multidrug resistance among ICU UPEC isolates. Continuous molecular surveillance is essential for effective infection control and antimicrobial stewardship.

Contributions of the author:

The author designed the study, collected and analysed the data, and wrote the manuscript for publication.

Source of funding:

No external funding was received

Conflict of interest:

No conflict of interest is declared

References:

1. World Health Organization (WHO). Global Action Plan on Antimicrobial Resistance. 2015.
2. World Health Organization (WHO). GLASS Report. 2022.
3. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats. 2019.
4. European Centre for Disease Prevention and Control (ECDC). Antimicrobial resistance surveillance. 2023.
5. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 31st Edition, CLSI Supplement M100, 2022
6. Goldstein, C., Lee, M. D., Sanchez, S., et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother. 2001; 45(3): 723–726. doi: 10.1128/AAC.45.3.723-726.2001
7. Cambray, G., Guerout, A. M., and Mazel, D. Integrons. Annu Rev Genet. 2010; 44: 141–166. doi: 10.1146/annurev-genet-102209-163504.
8. Hall, R. M., and Collis, C. M. Mobile gene cassettes and integrons. Mol Microbiol. 1995; 15: 593–600. https://doi.org/10.1111/j.1365-2958.1995.tb02 368.x
9. Gillings, M. R. Integrons: past, present, and future. Microbiol Mol Biol Rev. 2022; 78 (2): 257-277. https://doi.org/10.1128/mmbr.00056-13
10. Pitout, J. D. D., and Laupland, K. B. Extended-spectrum beta-lactamase-producing Enterobacteriaceae. Lancet Infect Dis. 2008; 8: 159–166. doi: 10.1016/S1473-3099(08)70041-0.
11. Paterson, D. L., and Bonomo, R. A. Extended-spectrum beta-lactamases. Clin Microbiol Rev. 2005; 18: 657–686. doi: 10.1128/CMR.18.4.657-686.2005
12. Partridge, S. R., Kwong, S. M., Firth, N., and Jensen, S. O. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018; 31(4): e00088-17. doi: 10.1128/CMR.00088-17
13. World Health Organization (WHO). GLASS Report. 2023.
14. European Centre for Disease Prevention and Control (ECDC). Antimicrobial resistance surveillance in Europe. 2024.

Abdullahi et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): xxxx https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2480. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Spectrum of bacterial uropathogens and their antibiotic susceptibility among patients with chronic kidney disease in a tertiary hospital, northcentral Nigeria

[1]Abdullahi, A. J., [2]Babaita, K. A., *[2]Ogunniyi, T. J., [3]Oyedepo, D. S., and [1]Akanbi II, A. A.

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, University of Ilorin, Ilorin, Nigeria
[2]Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria
[3]Department of Nephrology, University of Ilorin Teaching Hospital, Ilorin, Nigeria
*Correspondence to: josephtolulopeogunniyi@gmail.com

Abstract:

Background: Chronic kidney disease (CKD) is increasingly recognized as a global public health problem. CKD patients are prone to comorbidities, such as diabetes mellitus, urinary tract infection (UTI), hypertension, immune-suppression, anaemia, malnutrition, inflammation, and vitamin deficiencies. However, UTI is known to be the most significant cause of morbidity and mortality in CKD patients. Therefore, this study aimed to identify the spectrum of uropathogenic bacteria in patients with CKD at the University of Ilorin Teaching Hospital (UITH), Ilorin, and determine their susceptibility to selected antibiotics.
Methodology: The study is a descriptive cross-sectional design conducted among 157 patients with CKD recruited from the renal clinic of UITH between January and June, 2024. Clean-catch voided midstream urine samples were collected for culture on CLED and blood agar plates and incubated at 37°C for 24 hours. Microbial identification and antimicrobial susceptibility were performed on the pure colonies of isolated bacteria using the VITEK 2 Compact system. The data were analyzed for descriptive statistics.
Results: This study reported 22.9% (n=36) prevalence of bacterial uropathogens, with *Escherichia coli* (22.2%, n=8) being the most frequently isolated pathogen among the CKD patients. Antimicrobial susceptibility of the Gram-negative isolates showed high sensitivity to imipenem (85.7%, n=24), ceftazidime (64.3%, n=18), ceftriaxone (63.0%, n=17), piperacillin-tazobactam (57.1%, n=16), and nitrofurantoin (55.6%, n=15), but high resistance to gentamicin (53.6%, n=15), augmentin (51.9%, n=14), and ciprofloxacin (50.0%, n=14). Antimicrobial susceptibility of the Gram-positive isolates showed 100% sensitivity to linezolid and vancomycin, but high resistance to cefoxitin (60.0%, n=3) and clindamycin (42.9%, n=3).
Conclusion: This study showed a relatively high prevalence of UTI among CKD patients, and the implicated uropathogens show high susceptibility to the common antibiotics that are clinically accessible.

Keywords: Chronic Kidney Disease, Urinary Tract Infection, Uropathogens, Antimicrobials

Received Dec 25, 2025; Revised Feb 24, 2026; Accepted Feb 27, 2026

Spectre des uropathogènes bactériens et leur sensibilité aux antibiotiques chez les patients atteints d'insuffisance rénale chronique dans un hôpital de référence du centre-nord du Nigéria

[1]Abdullahi, A. J., [2]Babaita, K. A., *[2]Ogunniyi, T. J., [3]Oyedepo, D. S., et [1]Akanbi II, A. A.

[1]Département de Microbiologie Médicale et de Parasitologie, Faculté des Sciences Cliniques Fondamentales, Université d'Ilorin, Ilorin, Nigéria
[2]Département de Microbiologie Médicale et de Parasitologie, Centre Hospitalier Universitaire d'Ilorin, Ilorin, Nigéria
[3]Département de Néphrologie, Centre Hospitalier Universitaire d'Ilorin, Ilorin, Nigéria
*Correspondance : josephtolulopeogunniyi@gmail.com

Résumé:

Contexte: L'insuffisance rénale chronique (IRC) est de plus en plus reconnue comme un problème de santé publique

mondial. Les patients atteints d'insuffisance rénale chronique (IRC) sont sujets à des comorbidités telles que le diabète, les infections urinaires, l'hypertension, l'immunosuppression, l'anémie, la malnutrition, l'inflammation et les carences vitaminiques. Cependant, les infections urinaires constituent la principale cause de morbidité et de mortalité chez ces patients. Cette étude visait donc à identifier le spectre des bactéries uropathogènes chez les patients atteints d'IRC au Centre hospitalier universitaire d'Ilorin (UITH) et à déterminer leur sensibilité à certains antibiotiques.
Méthodologie: Il s'agit d'une étude descriptive transversale menée auprès de 157 patients atteints d'IRC recrutés à la clinique de néphrologie de l'UITH entre janvier et juin 2024. Des échantillons d'urine du milieu de jet, recueillis par la méthode du jet moyen, ont été mis en culture sur gélose CLED et gélose au sang, puis incubés à 37 °C pendant 24 heures. L'identification microbienne et l'antibiogramme ont été réalisés sur les colonies pures de bactéries isolées à l'aide du système VITEK 2 Compact. Les données ont été analysées statistiquement.
Résultats: Cette étude a rapporté une prévalence de 22,9% (n=36) d'uropathogènes bactériens, *Escherichia coli* (22,2%, n=8) étant le pathogène le plus fréquemment isolé chez les patients atteints d'IRC. La sensibilité aux antimicrobiens des isolats à Gram négatif a montré une forte sensibilité à l'imipénem (85,7%, n=24), à la ceftazidime (64,3%, n=18), à la ceftriaxone (63,0%, n=17), à la pipéracilline-tazobactam (57,1%, n=16) et à la nitrofurantoïne (55,6%, n=15), mais une forte résistance à la gentamicine (53,6%, n=15), à l'augmentin (51,9%, n=14) et à la ciprofloxacine (50,0%, n=14). L'étude de la sensibilité aux antimicrobiens des isolats à Gram positif a révélé une sensibilité de 100.0% au linézolide et à la vancomycine, mais une forte résistance à la céfoxitine (60,0%, n=3) et à la clindamycine (42,9%, n=3).
Conclusion: Cette étude a mis en évidence une prévalence relativement élevée d'infections urinaires chez les patients atteints d'insuffisance rénale chronique, et les uropathogènes impliqués présentent une forte sensibilité aux antibiotiques couramment utilisés en pratique clinique.

Mots-clés: Insuffisance rénale chronique, Infection urinaire, Uropathogènes, Antimicrobiens

Introduction:

The prevalence of CKD is estimated to be 13.4%, resulting in a major global health concern (1,2). Its rise to prominence as a significant global cause of disease and mortality underscores the importance of thorough study and intervention across populations (1,3). Diabetes mellitus, hypertension, obesity, and an aging population are all contributing causes to the global increase in CKD (4). However, there are regional differences in the frequency of CKD, with certain factors, including exposure to environmental chemicals and infections, playing a role (5).

According to recent comprehensive evaluations, the prevalence in sub-Saharan Africa (SSA) was between 10.1% and 13.9% (6,7). Additionally, West Africa was found to have the highest pooled frequency on the continent, at 16% (8). In Africa, CKD is typified by young patients, high morbidity, and early mortality. Ninety percent of CKD patients pass away within ninety days after beginning dialysis. Nigeria has a population of 180 million, yet little is known about the epidemiology of CKD in the general population (8). However, the prevalence of CKD in Nigeria varied from 2.5 to 26%, indicating a high prevalence of CKD overall and the need for additional research to determine the actual burden of CKD in Nigerians (8,9).

Nevertheless, due to the lack of affordability of the life-saving treatments of renal replacement therapies, which have been available for the past thirty years, the disease is silently killing a large number of Nigerians (10). Meanwhile, the majority of patients with CKD arrive late to nephrologists in Nigeria, already uremic and in need of renal replacement therapy (RRT) (11).

Similar to patients with other acquired immune deficits or those receiving immunosuppressive medication, patients with CKD and renal failure are at increased risk for infectious complications (12). Because metabolic diseases cause secondary immunological changes that impact numerous resistance components, chronic renal failure is therefore a risk factor for urinary tract infections (UTIs) (13,14). Haemodialysis may also weaken the immune system of CKD patients, leaving them more susceptible to illnesses like UTIs. Consequently, this could result in recurrent UTIs and kidney infections in the past (13,14).

Therefore, CKD could be a risk factor for UTIs, which can manifest as asymptomatic bacteriuria or symptomatic infection that requires treatment. A study in Nepal reported the prevalence of bacteria causing UTIs among CKD patients to be 15.8%, which was significantly high in the setting (15). UTI in CKD patients can occur as a mild case of cystitis, which can be treated with oral antibiotics, or a serious case of sepsis and multiple organ failures, and usually severe or potentially fatal infections manifest as complex UTI cases (16). If left undiagnosed or untreated, a UTI could result in significant deterioration in renal function with progress to advanced stages of CKD and death (15).

The prevalence of CKD in Nigeria is an indication for the investigation of UTI as a complication associated with CKD, in order to improve the quality of life of CKD patients. With paucity of data on the distribution of bacterial uropathogens and their susceptibility to commonly used antimicrobials among CKD patients

in Nigeria, this study aimed to determine the spectrum of bacterial uropathogens and their antibiotic susceptibility among CKD patients attending the University of Ilorin Teaching Hospital, Ilorin, Nigeria.

Materials and method:

Study setting:

The study was conducted at the University of Ilorin Teaching Hospital (UITH). The hospital was selected because it is the only tertiary healthcare facility in Ilorin serving large population with advanced health challenges and a referral centre for hospitals in Kwara, Oyo, Osun, Niger, Kogi, and Ekiti States in Nigeria.

Study design, sample size, and method of sampling:

The study was a descriptive cross-sectional design to determine the prevalence of bacterial uropathogens among patients with CKD and determine their antibiotic susceptibility. To determine the sample size, the prevalence of 10.1% of CKD reported in a study conducted in Sub-Saharan Africa (7) was incorporated into the formula (17); $N=Z^2 PQ(1-P)/d^2$, where N = minimum desired sample size, Z= normal standard deviation at 95% confidence interval (= 1.96), Q = 1-P, and d= precision (allowable error) of 5% (0.05), which gave a sample size of 138.

However, a total of 157 CKD patients were selected using consecutive sampling technique to recruit patients who satisfied the inclusion criteria and showed signs of a UTI during the period of January and June 2024, whether they were hospitalized in the nephrology unit or attended the renal clinic.

Inclusion and exclusion criteria:

Consenting CKD patients who willingly provided their urine samples and who had not been placed on antibiotics were included in the study. Patients' prescription history was assessed, and information on patients' medication history were further obtained to identify those who have not been on antibiotics. However, patients who have not been clinically diagnosed with CKD, CKD patients on antibiotics, and those who did not give consent to participate were excluded from the study.

Ethical consideration:

Ethical approval was obtained from the University of Ilorin Teaching Hospital Ethical Review Committee with the ERC approval number of ERC PAN/2023/05/0361 before commencing the project. All participants were informed about the purpose of the research, benefits, and risks, with certainty that their participation was voluntary and guaranteeing the confidentiality of data and their right to partake or withdraw from the study. All participants included in the study provided written informed consent to participate in the study.

Sample and data collection:

A midstream voided urine specimen of each CKD patient was collected into a sterile leak-proof universal bottle. Patients were properly instructed on how to collect clean catch urine. Samples were transported immediately for processing and analysis. Bio data of the sample, including specimen types, clinical diagnosis, gender, hospital unit, and age, was recorded in a format created especially for the research.

Laboratory analysis of urine samples:

Each urine sample was properly mixed and aseptically inoculated onto the CLED and blood agar plates to aid the growth of bacteria owing to the availability of the nutrient on the media. The inoculated agar plates were incubated at 37°C for 24 hours. Bacterial growth colonies were Gram-stained following standard procedure (18), and microbial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system.

Data analysis:

Data were analyzed using SPSS version 25.0. Descriptive statistics (chart, frequency, and percentage) were used to summarize the data.

Results:

Demographic characteristics of the participants:

Majority of the participants were females (54.8%, n=86), and in the age group above 60 years (37.6%, n=59), while the age group 1-10 years (1.3%, n=2) was the least frequent (Table 1).

Table 1: Socio-demographic characteristics of patients with chronic kidney disease in UITH Ilorin

Variables	Frequency	Percentage
Gender		
Male	71	45.2
Female	86	54.8
Age group (years)		
1-10	2	1.3
11-20	8	5.1
21-30	16	10.2
31-40	17	10.8
41-50	23	14.6
51-60	32	20.4
Above 60	59	37.6
Total	**157**	**100.00**

Prevalence of bacterial pathogens:

Of the total samples from 157 CKD participants, a prevalence of 22.9% (n=36) of bacterial uropathogens was reported (Fig 1). *Escherichia coli* was the most frequently isolated bacterium among the participants (22.2%, n=8), followed by *Klebsiella pneumoniae* (19.4%, n=7). *Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Streptococcus mutans*, *Acinetobacter* species, and *Enterococcus faecalis* were the least frequently isolated pathogens, with 2.8% (n=1) each (Table 2).

Antimicrobial susceptibility of bacterial uropathogens:

The antimicrobial susceptibility of the Gram-positive isolates showed 100% sensitivity to linezolid and vancomycin, but high resistance of 60.0% (n=3) and 42.9% (n=3) to cefoxitin and clindamycin, respectively (Table 3). The antimicrobial susceptibility of the Gram-negative bacteria showed sensitivity to imipenem (85.7%, n=24), ceftazidime (64.3%, n=18), ceftriaxone (63.0%, n=17), piperacillin-tazobactam (57.1%, n=16), and nitrofurantoin (55.6%, n=15), but the isolates were resistant to gentamicin (53.6%, n=15), augmentin (51.9%, n=14), and ciprofloxacin (50.0%, n=14) (Table 4).

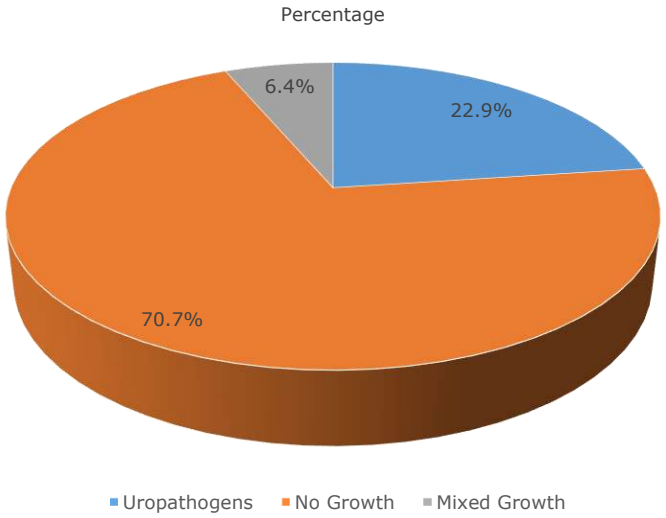


Fig 1: Prevalence of uropathogens among patients with chronic kidney disease in UITH Ilorin

Table 2: Frequency Distribution of bacterial uropathogens isolated from patients with chronic kidney disease in UITH Ilorin

Bacterial isolates	Frequency	Percentage
Gram-negative	**29**	**82.6**
Escherichia coli	8	22.2
Klebsiella pneumoniae	7	19.4
Klebsiella oxytoca	3	8.3
Enterobacter cloacae	3	8.3
Proteus mirabilis	3	8.3
Serratia ficaria	2	5.6
Pseudomonas aeruginosa	2	5.6
Acinetobacter specie	1	2.8
Gram-positive	**7**	**17.4**
Staphylococcus saprophyticus	2	5.6
Staphylococcus lugdunensis	1	2.8
Staphylococcus hominis	1	2.8
Staphylococcus lentus	1	2.8
Streptococcus mutans	1	2.8
Enterococcus faecalis	1	2.8
Total	**36**	**100.0**

Table 3: Antimicrobial susceptibility of Gram-positive isolates from patients with chronic kidney disease in UITH Ilorin

Antibiotics	No of isolates tested	No sensitive (%)	No intermediate (%)	No resistant (%)
Cefoxitin	5	2 (40)	0	3 (60)
Linezolid	7	7 (100)	0	0
Erythromycin	7	4 (57.1)	2 (28.6)	1 (14.3)
Clindamycin	7	4 (57.1)	0	3 (42.9)
Gentamicin	5	3 (60)	1 (20)	1 (20)
Tetracycline	5	4 (80)	0	1 (20)
Levofloxacin	5	3 (60)	2 (40)	0
Nitrofurantoin	7	6 (85.7)	0	1 (14.3)
Vancomycin	7	7 (100)	0	0

Table 4: Antimicrobial susceptibility of Gram-negative bacterial isolates from patients with chronic kidney disease in UITH Ilorin

Antibiotics	No of isolates tested	No sensitive (%)	No intermediate (%)	No resistant (%)
Ceftazidime	28	18 (64.3)	2 (7.1)	8 (28.6)
Augmentin	27	10 (37.0)	3 (11.1)	14 (51.9)
Ceftriaxone	27	17 (63.0)	0	10 (37.0)
Piperacillin-Tazobactam	28	16 (57.1)	0	12 (42.9)
Gentamicin	28	12 (42.9)	1 (3.6)	15 (53.6)
Ciprofloxacin	28	13 (46.4)	1 (3.6)	14 (50)
Imipenem	28	24 (85.7)	1 (3.6)	3 (10.7)
Nitrofurantoin	27	15 (55.6)	5 (18.5)	7 (25.9)

Discussion:

This study reported the prevalence of bacterial uropathogens to be 22.9%, which agrees with 26% rate reported by Chaudary et al., (19) but contrast the 70.6% rate reported by Raphael and Udo (20). This difference in rates may be due to differences in the regions and hospital settings of the studies. In patients with CKD, infectious complications are much higher, similar to individuals on immunosuppressive medications or those with other forms of acquired immune deficiency (12). The risk of infection in CKD patients has been reported to be three times higher than the general population (21). Also, the vulnerability of CKD patients to diseases such as UTI is due to prolonged haemodialysis, compromising their immune system function, resulting in recurrent UTIs and ascending renal infection. More so, chronic renal failure serves as a contributing factor for the development of UTIs due to secondary immunological changes brought on by metabolic diseases that impact several resistance components (13,14).

In this study, *E. coli* is the most frequently isolated bacterium in the CKD patients, contributing to about 22.2% of the total isolates, followed by *K. pneumoniae* (19.4%) and *K. oxytoca* (8.3%). This is similar to the studies of Pugalendhi et al., (22) and Shankar et al., (23), who both reported *E. coli* (47.7%) and *K. pneumoniae* (15.4%), and *E. coli* (61.8%) and *K. pneumoniae* (13.7%), respectively, as the most frequent bacterial isolates with a lower frequency of other bacteria uropathogen in CKD patients. This finding may be because *E. coli* is a ubiquitous bacterium and is the most predominant bacterium in UTI, hence a high tendency for *E. coli* to be reported in CKD patients. Also, numerous virulence factors, such as flagella, lipopolysaccharides, toxins, surface vesicles, polysaccharide capsules, fimbrial and non-fimbrial adhesins, and iron acquisition system, enable *E. coli* to survive and cause infection in the urinary tract. Thus, *E. coli* frequently targets the urethra, bladder, and kidneys (24).

Assessing the antibiotic susceptibility of the bacterial uropathogens, Gram-positive bacteria had higher susceptibility to linezolid and vancomycin compared to other antibiotics, while 60% and 42.9% of the isolates were resistant to cefoxitin and clindamycin, respectively. This finding is similar to that reported by Shankar et al., (23), with higher susceptibility of Gram-positive cocci to linezolid and vancomycin. This indicates the continued efficacy of these antibiotics against Gram-positive bacteria. This may partly be because these drugs cannot be bought easily over the counter, as they are mainly used in hospitals.

In this study, most of the Gram-negative bacteria showed high sensitivity to ceftazidime, ceftriaxone, piperacillin-tazobactam, imipenem, and nitrofurantoin, but were resistant to augmentin, gentamicin, and ciprofloxacin. This finding contrast that of Thapa et al., (25), who reported higher resistance of Gram-negative bacteria to ceftazidime and ceftriaxone, but agrees with their findings of high susceptibility to meropenem and nitrofurantoin. Our study rep-

ort absence of multi-drug resistance among the uropathogens from the CKD patients, which may be an important factor in management of the patients, with potential improved outcomes as resistance appears not to be a challenge.

This study has some limitations. We could not determine the distribution of isolated bacterial pathogens across the 5 stages of CKD, which would be beneficial in determining the stages that are most at risk of UTI. Additionally, the study did not take into account the risk factors that influence the prevalence of UTI. However, the study leverages the strength of the automated method (Vitek 2 system) used for bacteria identification and susceptibility testing.

Conclusion:

This study reported 22.9% prevalence of uropathogenic bacteria, with *E. coli* being the predominant pathogen among CKD patients. Bacterial uropathogens isolated from CKD patients in this study show high susceptibility to the common antibiotics that are clinically accessible, indicating that antimicrobial resistance is not significantly high among CKD patients. Future research should explore the association between the stages of CKD and UTIs, and other risk factors should be considered.

Acknowledgements:

The authors acknowledge the staff of the department of medical microbiology and parasitology, and the staff of the renal centers, for their support and guidance through the completion of the study. The guidance of Professor G.P. Oyeyiola, Dr. Aliyu Badadoko, and Mr. Bello Adegoke is acknowledged.

Contributions of authors:

AJA conceptualized the idea and designed the study, and was involved in data acquisition, interpretation and writing of the manuscript; KAB was involved in data interpretation, validation and writing of the manuscript; TJO was involved in data analysis and interpretation, writing, revising and proofreading of the manuscript; DSO was involved in data interpretation and validation, reviewing and proofreading of the manuscript and AAAII supervised the study and was involved in conceptualization, design, data acquisition and interpretation, reviewing and proofreading of the manuscript. All authors approved the final version of the manuscript submitted for publication.

Source of funding:

No external funding was received

Conflict of interest:

Authors declare no conflict of interest

References:

1. Lv, J-C., and Zhang, L-X. Prevalence and Disease Burden of Chronic Kidney Disease. In: Advances in Experimental Medicine and Biology. Springer Singapore; 2019: 3-15. http://dx.doi.org/10.1007/978-981-13-8871-2_1
2. Kovesdy, C. P. Epidemiology of chronic kidney disease: an update 2022. Kidney International Supplements. 2022; (1): 7-11. doi:10.1016/j.kisu.2021.11.003
3. Lankinen, R., Hakamäki, M., Metsärinne, K., et al. Cardiovascular Determinants of Mortality in Advanced Chronic Kidney Disease. Am J Nephrol. 2020; 9: 726-735. doi:10.1159/000509582
4. Smyth, L. J., Duffy, S., Maxwell, A. P., and McKnight, A. J. Genetic and epigenetic factors influencing chronic kidney disease. Am J Physiol -Renal Physiol. 2014; 7: F757-F776. doi:10.1152/ajprenal.00306.2014
5. Friedman, D. J. Genes and environment in chronic kidney disease hotspots. Curr Opinion Nephrol Hypert. 2019; 1: 87-96. doi:10.1097/mnh.0000000000000470
6. Stanifer, J. W., Jing, B., Tolan, S., et al. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. Lancet Glob Health. 2014; 3: e174-e181. doi:10.1016/s2214-109x(14)70002-6
7. Abd ElHafeez, S., Bolignano, D., D'Arrigo, G., Dounousi, E., Tripepi, G., and Zoccali, C. Prevalence and burden of chronic kidney disease among the general population and high-risk groups in Africa: a systematic review. BMJ Open. 2018; 1: e015069. doi:10.1136/bmjopen-2016-015069
8. Olanrewaju, T. O., Aderibigbe, A., Popoola, A. A., et al. Prevalence of chronic kidney disease and risk factors in North-Central Nigeria: a population-based survey. BMC Nephrol. 2020; 1. doi:10.1186/s12882-020-02126-8
9. Chukwuonye, I. I., Ogah, O., Anyabolu, E., et al. Prevalence of chronic kidney disease in Nigeria: systematic review of population-based studies. Int J Nephrol. Renovasc Dis. 2018: 165-172. doi:10.2147/ijnrd.s162230
10. Ibitoba, F. A., Akpor, O. A., and Akpor, O. B. Prevalence and risk factors of chronic kidney disease among commercial motorcyclists in Ado-Ekiti, Ekiti State, Nigeria. Sci Afr. 2022: e01136. doi:10.1016/j.sciaf.2022.e01136
11. Adejumo, O., Akinbodewa, A., Okaka, E., Alli, O., and Ibukun, I. Chronic kidney disease in Nigeria: Late presentation is still the norm. Nig Med J. 2016; 3: 185. doi:10.4103/0300-1652.184072
12. Cristea, O. M., and Avrămescu, C. S. Urinary tract infection with *Klebsiella pneumoniae* in Patients with Chronic Kidney Disease. Curr Health Sci J. 2017; 2: 137-148. doi:10.12865/CHSJ.43.02.06
13. Scherberich, J. E., Fünfstück, R., and Naber, K. G. Urinary tract infections in patients with renal insufficiency and dialysis – epidemiology, pathogenesis, clinical symptoms, diagnosis and treatment. 2021. doi:10.3205/ID000076
14. Dicu-Andreescu, I., Penescu, M. N., Căpușă, C., and Verzan, C. Chronic Kidney Disease, Urinary Tract Infections and Antibiotic Nephrotoxicity: Are There Any Relationships? Medicina. 2022; 1: 49. doi:10.3390/medicina59010049

15. Almaiman, L., Allemailem, K. S., El-Kady, A. M., et al. Prevalence and Significance of Pyuria in Chronic Kidney Disease Patients in Saudi Arabia. J Personalized Med. 2021; 9: 831. doi:10.3390/jpm11090831
16. Dimitrijevic, Z., Paunovic, G., Tasic, D., Mitic, B., and Basic, D. Risk factors for urosepsis in chronic kidney disease patients with urinary tract infections. Sci Rep. 2021; 1. doi:10.1038/s41598-021-93912-3
17. Charan, J., and Biswas, T. How to Calculate Sample Size for Different Study Designs in Medical Research? Indian J Psychol Med. 2013; 2: 121-126. doi:10.4103/0253-7176.116232
18. Cheesbrough, M. Laboratory Practice in Tropical Countries. Part II. Cambridge, England: Cambridge University Press; 2012.
19. Chaudhary, R. Bacteriology of Urinary Tract Infection of Chronic Renal Failure Patients Undergoing for Haemodialysis. J Microbiol Exp. 2016; 3. doi:10.15406/jmen.2016.03.00089
20. Raphael, J. E., and Udo, K. Bacteriological Spectrum of Urine Culture in Patients with Obstructive Uropathy. Am J Med Sci Med. 2022; 10 (1): 18-22. doi:10.12691/ajmsm-10-1-3
21. Eshwarappa, M., Dosegowda, R., Aprameya, I., Khan, M., Kumar, P, and Kempegowda, P. Clinico-microbiological profile of urinary tract infection in South India. Indian J Nephrol. 2011; 1: 30. doi:10.4103/0971-4065.75226
22. Pugalendhi, S., Dutta, T. K., R. H, S. L. A clinical and microbiological profile of urinary tract infection in patients with chronic kidney diseases. Int J Adv Med. 2019; 3: 911. doi:10.18203/2349-3933.ijam20192263
23. Shankar, M., and Narasimhappa, S, N. S. M. Urinary Tract Infection in Chronic Kidney Disease Population: A Clinical Observational Study. Cureus. 2021. doi:10.7759/cureus.12486
24. Zhou, Y., Zhou, Z., Zheng, L., et al. Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. Int J Mol Sci. 2023; 13: 10537. doi:10.3390/ijms241310537
25. Thapa, T. B., Pokhrel, S., Lamichhane, A., et al. Prevalence and antibiogram of bacteria causing urinary tract infection among patients with chronic kidney disease. Open Med. 2023; 1. doi:10.1515/med-2023-0824

Usang et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 402 - 405 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2498. https://www.ajol.info/index.php/ajcem

Short Communication **Open Access**

Prevalence and identification of microbial contaminants of reusable plastic containers from informal food vending sites in Calabar, Nigeria

*[1]Usang, A., [2]Omeh, B. A., and [3]Ekuma, V. O.

[1]Faculty of Medical Laboratory Science, University of Calabar, PMB 1115, Calabar, Nigeria
[2]Department of Medical Laboratory Science, College of Health Technology, Calabar, Nigeria
[3]Department of Parasitology and Entomology, University of Calabar, Nigeria
*Correspondence to: akedorusang2rue@gmail.com

Abstract

Background: Reusable plastic containers are widely used in informal food vending sites in developing countries like Nigeria, posing potential risks for microbial contamination and foodborne illnesses. This study determined the prevalence and identify microbial contaminants in 350 reusable plastic containers sampled from selected sites (markets, schools, roadside sellers, hawkers, and motor parks) in Calabar, Nigeria.
Methodology: The study applied standard swab and culture method to collect and analyze samples, which involved sampling 350 different containers from the 5 randomly selected sampling sites in Calabar. Chi-square test was used to assess the differences in contamination rates of containers between the sampling sites with $p<0.05$ considered as statistical significance level.
Results: Overall, 288 (82.3%) containers were contaminated, predominantly with bacteria (81.7%, n=286), which includes *Escherichia coli* (56.6%, n=163), *Staphylococcus aureus* (37.4%, n=108), *Enterococcus faecalis* (4.2%, n=12), and *Pseudomonas aeruginosa* (1.1%, n=3). Fungal contaminants were minimal (0.6%, n=2), with no parasites detected. Contamination rates varied by sites but showed no significant differences between sampling sites ($\chi^2=7.40$, $p=0.116$).
Conclusion: These findings highlight significant public health risks, comparable to high contamination levels in other African contexts but exceeding those in developed nations. Interventions focusing on hygiene education and regulation are recommended to mitigate foodborne disease burdens in Nigeria.

Keywords: Microbial contamination, reusable plastic containers, food vending, pathogens, Nigeria

Received Feb 14, 2026; Revised Apr 30, 2026; Accepted May 1, 2026

Prévalence et identification des contaminants microbiens des contenants en plastique réutilisables provenant de points de vente alimentaires informels à Calabar, au Nigéria

*[1]Usang, A., [2]Omeh, B. A., et [3]Ekuma, V. O.

[1]Faculté des Sciences de Laboratoire Médical, Université de Calabar, PMB 1115, Calabar, Nigéria
[2]Département des Sciences de Laboratoire Médical, École Supérieure de Technologie de la Santé, Calabar, Nigéria
[3]Département de Parasitologie et d'Entomologie, Université de Calabar, Nigéria
*Correspondance à: akedorusang2rue@gmail.com

Résumé:

Contexte: Les contenants en plastique réutilisables sont largement utilisés dans les points de vente alimentaires informels des pays en développement comme le Nigéria, ce qui représente un risque potentiel de contamination microbienne et de maladies d'origine alimentaire. Cette étude a déterminé la prévalence et identifié les contaminants microbiens présents dans 350 contenants en plastique réutilisables prélevés dans différents sites (marchés, écoles,

vendeurs ambulants, colporteurs et gares routières) à Calabar, au Nigéria.
Méthodologie: L'étude a utilisé la méthode standard de prélèvement et de culture pour collecter et analyser les échantillons. L'échantillonnage a porté sur 350 récipients différents provenant de 5 sites sélectionnés aléatoirement à Calabar. Le test du χ^2 a été utilisé pour évaluer les différences de taux de contamination des récipients entre les sites d'échantillonnage, le seuil de signification statistique étant fixé à $p<0,05$.
Résultats: Au total, 288 récipients (82,3%) étaient contaminés, principalement par des bactéries (81,7%, n=286), notamment *Escherichia coli* (56,6%, n=163), *Staphylococcus aureus* (37,4%, n=108), *Enterococcus faecalis* (4,2%, n=12) et *Pseudomonas aeruginosa* (1,1%, n=3). La contamination fongique était minime (0,6%, n=2) et aucun parasite n'a été détecté. Les taux de contamination variaient selon les sites, mais aucune différence significative n'a été observée entre les sites d'échantillonnage ($\chi^2=7,40$, $p=0,116$).
Conclusion: Ces résultats mettent en évidence des risques importants pour la santé publique, comparables aux niveaux de contamination élevés observés dans d'autres contextes africains, mais supérieurs à ceux des pays développés. Des interventions axées sur l'éducation et la réglementation en matière d'hygiène sont recommandées afin de réduire la charge des maladies d'origine alimentaire au Nigéria.

Mots-clés: Contamination microbienne, contenants en plastique réutilisables, vente ambulante d'aliments, agents pathogènes, Nigéria

Introduction:

Food safety remains a critical public health challenge in developing countries, where informal food vending contributes significantly to dietary intake but often lacks stringent hygiene standards (1). In Nigeria, street-vended foods are a staple for urban populations, yet they are frequently associated with microbial contamination due to poor handling practices, inadequate sanitation, and reuse of containers without proper cleaning (2). Reusable plastic containers, common in informal settings, can harbor pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*, leading to foodborne illnesses that account for over 200,000 deaths annually in Nigeria (3).

Globally, the World Health Organization estimates 600 million cases of foodborne diseases yearly, with sub-Saharan Africa bearing a disproportionate burden (4). In Calabar, a bustling city in southern Nigeria, informal vending sites like markets and motor parks serve as hubs for food distribution, exacerbating contamination risks through environmental exposure and cross-contamination (5). Previous studies in Nigeria have reported high bacterial loads in food contact surfaces, but data specific to reusable containers in Calabar is limited (6).

The objectives of this study are to determine the prevalence of microbial contaminants, identify bacterial and fungi isolates, and assess variations across vending sites, with the aim of providing insights for targeted interventions.

Materials and method:

Study design, sampling sites and collection:

A cross-sectional study was conducted in Calabar, Nigeria, from January to June 2025. A total of 350 reusable plastic containers were randomly sampled from five informal food vending sites; markets (n=126), schools (n=74), roadside sellers (n=84), hawkers (n=52), and motor parks/garages (n=14). Containers were selected based on active use for food storage or serving.

Sterile swabs moistened with buffered peptone water were used to sample inner surfaces, following standard protocols (ISO 18593: 2018). Swabs were transported in cool boxes to the laboratory within 2 hours for analysis.

Microbial culture, isolation and identification:

Swabs were inoculated into enrichment broth (tryptic soy broth) and incubated at 37°C for 24 hours. For bacterial isolation, serial dilutions were plated on selective media; MacConkey agar for *E. coli*, mannitol salt agar for *S. aureus*, *Pseudomonas* cetrimide agar for *P. aeruginosa*, and bile esculin azide agar for *Enterococcus* (7).

Colonies were enumerated as colony-forming units (CFU/ml) and identified by Gram stain reaction and biochemical tests (API 20E systems) (8). Fungal isolation involved culture on Sabouraud dextrose agar (with chloramphenicol), incubation at 25°C for 5-7 days, followed by microscopic identification. Parasites were identified in urine samples by wet mounts and concentration techniques (9).

Statistical analysis:

Data obtained from the laboratory culture, isolation and identification were analyzed using SPSS version 26.0. Contamination rates were expressed as percentages. Chi-square test was used to determine associations between sites and contamination rates, with $p<0.05$ considered statistical significance.

Results

Of the 350 containers sampled, 288 (82.3%) were contaminated with microbial pathogens (Table 1). There is no significant diffe-

rence in contamination rates between the five different sampling sites (χ^2=7.40, df=4, p= 0.116) (Table 2).

Bacterial contamination predominated, in 286 containers (81.7%), fungal in 2 (0.6%), with 2 overlaps (0.6%), but no parasite was detected in any container (Table 3). Bacterial isolates included *E. coli* (56.6%, n=163), *S. aureus* (37.4%, n=108), *E. faecalis* (4.2%, n=12), and *P. aeruginosa* (1.1%, n=3) (Table 4).

Table 1: Contamination rate of food vending plastic containers in Calabar, Nigeria

Parameter	Number	Percentage (%)
No of containers sampled	350	-
No of containers contaminated	288	82.3
No of containers uncontaminated	62	17.7

Table 4: Contamination by sites

Site	No sampled	No contaminated	%
Markets	126	105	83.3
Schools	74	64	86.5
Roadside sellers	84	67	79.8
Hawkers	52	38	73.1
Motor parks	14	14	100.0
Total	350	288	82.3

No significant site differences (χ^2=7.40, df=4, p=0.116)

Table 3: Contamination of containers by microbial types in Calabar, Nigeria

Type	Number	Percentage (%)
Bacterial	286	81.7
Fungal	2	0.6
Parasitic	0	0
Bacterial + Fungal	2	0.6

Table 3: Frequency of bacterial isolates contaminating food vending plastic containers in Calabar, Nigeria

Species	Number of isolates	Percentage (%)
Escherichia coli	163	56.6
Staphylococcus aureus	108	37.4
Enterococcus faecalis	12	4.19
Pseudomonas aeruginosa	3	1.05
Total	**286**	**100.0**

Discussion:

The high contamination rate (82.3%) observed underscores the vulnerability of reusable plastic containers in informal vending, aligning with studies in Nigeria reporting 70%-90% bacterial loads in similar surfaces (6,9). This high contamination rate can be attributed to poor quality water used in these informal food vending areas (10,11). *Escherichia coli* and *S. aureus* dominance reflects fecal and human skin sources, common in unhygienic handling (2), contrary to the study reporting *S. aureus* as the most prevalent bacteria isolated (12).

In Africa, comparable rates include 54.3% *E. coli* in Ethiopian street foods (13) and 37.6% in raw foods across the continent (14). Motor parks showed 100% contamination, likely due to high traffic and poor sanitation, similar to Mozambican vendors (15). Globally, developed countries report lower rates (e.g. <20% in U.S. food surfaces) due to strict regulations and advanced cleaning (7). In contrast, developing nations face higher burdens from inadequate infrastructure (1).

Microbial contamination in reusable food containers leads to economic losses through three main effects, which include creating unnecessary food waste and harming street vendor businesses and raising medical expenses and control expenses (16). This study's findings imply potential for outbreaks, contributing to Nigeria's $3.6 billion annual foodborne illness costs (3). Compared to Africa with 91 million cases/year, figures are lower in Europe/Asia due to better surveillance (4). Recommendations include vendor training, as in Ghanaian interventions reducing contamination by 40% (5). Limitation of this study include lack of molecular typing. Future research should incorporate AMR profiling.

Conclusion:

This study showed that reusable plastic containers used by informal food vendors showed high contamination rate of 82.3% probably due to unsatisfactory hygiene standards and unsafe water sources. The presence of the bacteria contaminants such as *E. coli* signifies fecal contamination from human excretions which poses high risk of foodborne diseases to people who live in high-density regions such as motor parks. The contamination of the containers sampled presents significant threats to public health that call for urgent measures to improve hygiene practices, vendor education programmes and effective food safety regulations.

Contributions of authors:

AU conceived the study and participated in the laboratory analysis; EVO edited, proof read and participated in the laboratory analysis; OBA analyzed the data collected. All authors approved the manuscript submitted for publication.

Source of funding:

No funding was received for the study

Conflict of interests:

Authors declare that they have no competing interests, financial or otherwise, that could have influenced the work reported in this manuscript

References:

1. Grace, D., Dipeolu, M., and Alonso, S. Improving food safety in the informal sector: nine years later. Infect Ecol Epidemiol. 2019; 9 (1): 1579613. doi: 10.1080/20008686.2019.1579613
2. Adesokan, H. K., Obimdike, O. C., and Adetunji, V. O. Informal and formal meat marketing in Ibadan, Nigeria: public health implications from microbial assessment. PAMJ-One Health. 2021; 5 (15). doi:10.11604/pamj-oh.2021.5.15.28743
3. Jimoh, O., Abdulkadir, M. I., Yusuf, T. I., et al. Bacterial Contaminants Associated with the Hands of Food Handlers at Ahmadu Bello University, Zaria. UMYU J Microbiol Res. 2021; 6 (1): 56-61. doi:10.47430/ujmr.2161.007
4. World Health Organization. Food Safety. 2022. https://www.who.int/news-room/fact-sheets/detail/food-safety (Accessed June 5, 2023).
5. Christiana Cudjoe, D., Balali, G. I., Titus, O. O., Osafo, R., and Taufiq, M. Food safety in sub-Sahara Africa, an insight into Ghana and Nigeria. Environ Health Insights. 2022; 16: 11786302221142484. doi: 10.1177/11786302221142484
6. Seiyaboh, E. I., Angaye, T. C., and Seiyaboh, Z. Assessment of Microbiological Contaminants Associated with Locally Processed Beverages in Reused Plastic Container. Direct Res J Publ Health Environ Technol. 2020; 5 (4): 49-51. doi: 10.26765/DRJPHT8037126915
7. Food and Drug Administration. Food Code. 2024 Recommendations of the United States Public Health Service. https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm374275.htm. (Accessed 5 June 2024)
8. Cheesbrough, M. District laboratory practice in tropical countries. Cambridge University Press, England; 2022
9. Umar, A. T., Haruna, A., Puma, H. U., et al. Bacterial Contaminants of New Unused Disposable Food Packs Used in Commercial Area of Gombe State University. South Asian J Res Microbiol. 2021; 10 (3): 1-6. doi:10.9734/sajrm/2021/v10i330228
10. Sun, X., and Kim, J. The Cleanliness of Reusable Water Bottles: How Contamination Levels are Affected by Bottle Usage and Cleaning Behaviors of Bottle Owners. Food Protection Trends. 2017; 37 (6):
11. Abrokwah, S., Ekumah, B., and Abrokwah, F. K. Microbial assessment of plastic bottles reused for packaging food products in Ghana. Food Contr. 2020; 109: 106956. doi: 10.1016/j.foodcont.2019.106956
12. Edeh, P. A., Ekuma, U. O., Ede, J. U., et al. Detection and profiling of antibiotic resistance among bacteria isolates from new and unused styrofoam and single use plastics sold in Orlu, Imo State Nigeria. Health. 2025; 4: 5. doi: 10.30574/gscbps.2025.30.2.0027
13. Abebe, E., Gugsa, G., Ahmed, M., et al. Occurrence and antimicrobial resistance pattern of E. coli O157: H7 isolated from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. PLoS Negl Trop Dis. 2023; 17 (1): e0010706. doi: 10.1371/journal.pntd.0010706
14. Traore, K. A., Sanou, M., Ouoba, J. B., Ouoba, B. L., Roques, P., and Barro, N. Detection of adenovirus in fresh fruit, vegetables, wastewater and manure from irrigated farms in Ouagadougou, Burkina Faso. Food Nutr Sci. 2024; 15 (7): 644-662. doi: 10.4236/fns.2024.157041
15. Salamandane, A., Silva, A. C., Brito, L., and Malfeito-Ferreira, M. Microbiological assessment of street foods at the point of sale in Maputo (Mozambique). Food Qual Safety. 2021; 5: fyaa030. doi: 10.1093/fqsafe/fyaa030
16. Licciardello, F. Unexpected possible consequences of plastic packaging reuse. Curr Opinion Food Sci. 2024; 56: 101131. doi: 10.1016/j.cofs.2024.101131

ERRATUM

Ramdeen et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 406 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2467. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Discovery of potential antiviral compounds against Dengue virus using virtual screening and physicochemical profiling from zinc database

[1]Ramdeen, S. J., [1]Usoro, N., [1]Giordano, K., [1]Garza, P. E., [2]Quadri, O., [3]Esan, T. E., and [*1]Olanrewaju, A. A.

[1]Texas State University, Medical Laboratory Science Program San Marcos, TX USA
[2]Northern Illinois University, Chemistry and Biochemistry Analytical Lab, Dekalb, IL USA
[3]Rosalind Franklin University of Medicine and Science, Chicago, IL USA
[*]Correspondence to: dep112@txstate.edu; Phone: +1 571-228-0537

Erratum to: Afr. J. Clin. Exper. Microbiol. 2026; 27 (2): 126-133. https://dx.doi.org/10.4314/ajcem.v27i2.1

A publication error in the name of author, Quadri, P., in the previously published version of this article has been appropriately corrected as Quadri, O., in this authors page. All information in the previously published article remained valid.

Découverte de composés antiviraux potentiels contre le virus de la dengue par criblage virtuel et profilage physico-chimique à partir d'une base de données sur le zinc

[1]Ramdeen, S. J., [1]Usoro, N., [1]Giordano, K., [1]Garza, P. E., [2]Quadri, O., [3]Esan, T. E., et [*1]Olanrewaju, A. A.

[1]Université d'État du Texas, Programme de Sciences Laboratoire Médical, San Marcos, Texas, États-Unis [2]Université du Nord de l'Illinois, Laboratoire d'Analyse de Chimie et Biochimie, Dekalb, Illinois, États-Unis [3]Université de Médecine et Sciences Rosalind Franklin, Chicago, Illinois, États-Unis
*Correspondance à: dep112@txstate.edu; Tél: +1 571-228-0537

Une erreur de publication concernant le nom de l'auteur, Quadri, P., dans la version précédente de cet article, a été corrigée en Quadri, O., dans la présente page d'auteurs. Toutes les informations contenues dans la version précédente restent valides.

CORRIGENDUM

Olatunji et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 407 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2450. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Prevalence and predictors of hepatitis B screening among pregnant women attending antenatal clinics in three primary health centres in Abeokuta, Nigeria: A cross-sectional study

*Olatunji, O. A., Fowotade, A., and Omoruyi, E. C.

Department of Medical Microbiology, University College Hospital (UCH), Ibadan, Nigeria
*Correspondence to: aliu_my2004@yahoo.com

Corrigendum to: Afr. J. Clin. Exper. Microbiol. 2026; 27 (1): 46-53 https://dx.doi.org/10.4314/ajcem.v27i1.7

An error by the authors in a previously published version of this article with respect to arrangement of authors names, has been appropriately corrected in this authors page. All information in the previously published article remained valid.

Prévalence et facteurs prédictifs du dépistage de l'hépatite B chez les femmes enceintes fréquentant les consultations prénatales de trois centres de santé primaires à Abeokuta, au Nigéria: une étude transversale

*Olatunji, O. A., Fowotade, A., et Omoruyi, E. C.

Département de Microbiologie Médicale, Centre Hospitalier Universitaire (CHU), Ibadan, Nigéria
*Correspondance à: aliu_my2004@yahoo.com

Une erreur concernant l'ordre des noms des auteurs dans une version antérieure de cet article a été corrigée sur la page des auteurs. Toutes les informations contenues dans la version précédente restent valides.

ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (AJCEM) ISSN 1595-689X

https://www.ajol.info/index.php/ajcem and https://www.afrjcem.org

GENERAL INFORMATION

Aims and scope

African Journal of Clinical and Experimental Microbiology (AJCEM) is the official Journal of the African Society for Clinical Microbiology and published by AJCEM Lifeline RC 651882. It publishes original research, review papers, case reports/series, short communications and letters to the editors, in all aspects of Medical Microbiology including Bacteriology, Virology, Rickettsiology and Chlamydiology, Mycology, Mycobacteriology and Actinomycetes, Parasitology, Molecular Genetics in relation to microorganisms and humans, Clinical Microbiology, Clinical Veterinary Microbiology, and Public Health Microbiology.

Subscription information

Free downloads can be made from the website of the world's largest online library of peer reviewed, Africa-published scholarly journals, African Journals OnLine (AJOL): https://www.ajol.info/index.php/ajcem OR from the Journal website https://www.afrjcem.org. Subscription is however still open to individuals, libraries, University Departments, Research Institutes and other Multi-reader institutions who may want to have hard copies of the Journal. For each volume (4 issues), subscription rate is £400 (United Kingdom), US $800 (USA/Canada), US $600 (African Countries), US $800 (Other Countries), ₦28,000 (Nigeria). Additional charges will be made for postage and packaging. A copyright for these is with African Journal of Clinical and Experimental Microbiology.

Subscription enquiries and all other matters relating to the Journal including manuscripts, adverts booking and sponsorship should be addressed to:

Prof Samuel S. Taiwo (MD; FMCPath)
Editor-in-Chief, African Journal of Clinical and Experimental Microbiology,
Department of Medical Microbiology, LAUTECH Teaching Hospital,
PMB 4007, Ogbomoso, Nigeria
Mobile Phone: +234 (0) 8033436344
Email: afrjcem@gmail.com

Indexing and Abstracting Information

African Journal of Clinical and Experimental Microbiology is abstracted/indexed at the following bodies; (i) African Journals Online (AJOL) https://www.ajol.info/ with TWO STAR rating (out of a possible three) in the Journal Publishing Practices and Standards (JPPS) Framework assessment https://www.journalquality.info (March 2019); (ii) Index Copernicus International (ICI) Journal Master List www.indexcopernicus.com (ID 35531) with Index Copernicus Value (ICV) for 2019 of 93.10, and for 2020 of 71.43; (iii) Society of African Journal Editors (SAJE) Library https://africaneditors.org; with 2020 Journal Ranking of 'A', Impact Score of 1.092, h5 Index of 6, Citation of 119; and 2021 Journal Ranking of 'A', Impact Score of 1.143, h5 Index of 6, and Citation of 154; (v) African Researchers (AR) Index (https://www.africanresearchers.org/arindex/african-journal-of-clinical-and-experimental-microbiology/)

Guidelines to authors/contributors

It is a condition of publication that manuscripts submitted to this Journal have not been published and will not be simultaneously submitted to be published elsewhere except as conference abstracts, for which authors must disclose at the point of manuscript submission. Authors should be aware that electronic journals issues/articles can be accessed free (Open Access) online at the AJOL website: https://www.ajol.info/index.php/ajcem OR at the Journal website: https://www.afrjcem.org

Responsibility for accuracy of manuscripts lies entirely with the authors. All submissions must conform to the International Committee of Medical Journal Editors (ICMJE) uniform recommendations for manuscripts submitted to biomedical journals (http://www.icmje.org/recommendations/) and follow the guidelines of Committee on Publication Ethics https://publicationethics.org/guidance/Guidelines.

Manuscripts should be typewritten with double line spacing and wide margins, following the conventional form: Title, Authors' names in agreed order of arrangement, full address of the corresponding author including e-mail, phone number and ORCiD number (if available), Abstract (Structured), Introduction, Materials and Methods, Results, Discussion, Acknowledgment(s), References, Tables, Figures and Legends to Figures. Short Communications and Letters to The Editor are also entertained, but need not follow the above format.

ISSN 1595-689X

French abstracts:

The Journal has, since May 2014, be including French translations of abstracts. Authors should therefore include French translations of their titles, their addresses and abstracts of their manuscripts. If they are not included, it will be done by the Journal at a nominal fee to be paid as part of handling charges.

If the research involves the use of human subjects, including collection of human blood or other human specimens, an institutional ethical clearance document should be submitted with the manuscripts. Alternatively, a statement should be made in the "Materials and Methods" section that informed consent of the experimental subjects and the approval of the appropriate ethical committee had been obtained.

All necessary illustrations should accompany the manuscripts, but should not be in the text. The illustrations should be numbered consecutively in the order in which they are referred to in the text. The top of illustration should also be indicated if this is not clear. All x-ray films must be clear and should be in photographic prints. Legends to figures should give sufficient information to make the illustration comprehensive without reference to the text.

References should be listed in their order of appearance in the text; and be indicated in the text by Arabic numbers in brackets e.g. (1), (2, 3, 4), etc (Modified Vancouver style). Accuracy of the references is the responsibility of the authors. The authors' names and initials should be followed by the title of the paper, abbreviated name of the journal, which should conform to those used in Index Medicus, year of publication, volume, and the first and last page numbers. **The digital object identifier (DOI) number of each cited article should be included if available**. Note the following examples.

For Journals:

1. Nsanze, H. Recommendation for management of gonorrhoea and genital ulcers in Africa. Afr J Sex Transm Dis. 1984; 1:5-7

2. Odugbemi, T. O., Akinsulire, O. R., Aibinu, I. E., and Fabeku, P. O. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. Afr J Tradit Complement Altern Med. 2006; 13; 4 (2): 191-198.
doi: 10.4314/ajtcam.v4i2.31207

3. Bakare, R. A., Oni, A. A., Okesola, A. A., et al. Efficacy of pefloxacin on acute uncomplicated gonococcal urethritis. Nig Qt J Hosp Med. 1996; 6: 335

For books:

1. Arya, O. P., Osoba, A. O., and Bennett, P. Tropical Venereology, Churchill Livingstone, Edinburgh, 1980

When referring to a chapter in a book and where the names of authors are also given, the reference should be;

2. Easmon, C. S. F. Host-Parasite relationship in experimental staphylococcal infections. In: Macdonald, A., and Smith, G. (eds). The Staphylococci. University Press, Aberdeen 1981: 63-72

Copyright Notice

Authors who publish with AJCEM must agree to the following terms;

1. Authors transfer the copyright to AJCEM who publishes under CC BY license that allows authors and others to share the work with an acknowledgement of the works' authorship and initial publication in AJCEM

2. Authors are able to enter into separate, additional contractual arrangements for the non-exclusive and non-commercial distribution of AJCEM published version of the work (e. g. posting it on institutional repository or publishing it in a book), with acknowledgement of its initial publication in AJCEM

3. Authors are permitted and encouraged to post their work online (e. g. institutional repositories or on their websites) prior to or during the submission process as it can lead to productive exchanges as well as earlier and greater citation of published work

4. Authors must agree to the terms of this Copyright Notice, which will apply to the submitted manuscript if and when it is accepted and published by AJCEM. This agreement should be stated within the text of the manuscript at the time of submission.

Plagiarism Prevention Policy

The rights of all authors who publishes with AJCEM are protected and the integrity of the Journal is upheld in the highest possible form. Issues of copyright infringement, plagiarism and other breaches of publication best practices are carefully handled and investigated. All submitted manuscripts are checked with plagiarism software. Where there are errors in the published articles or where issues of plagiarism or copyright infringement are detected by the Journal or allegation of impropriety or authorship of a manuscript is contested, the Journal reserve the right to take actions which may include but not limited to; publishing an erratum (for publication errors) or corrigendum (for authors errors); retraction of the article; reporting cases of malpractices to heads of authors institutions and/or relevant academic bodies or societies; or instituting appropriate legal action.

ISSN 1595-689X

Manuscript submission

All manuscripts should preferably be submitted through the Journal's website at "Submit Your Manuscript" page (https://afrjcem.org/submit/) but alternatively, manuscripts could be submitted through the e-mail of the Journal (afrjcem@gmail.com)

Peer Review Process

The peer review process of the Journal can be accessed on the Journal's website at https://afrjcem.org/ajcem/. All manuscripts submitted to the Journal are first scrutinized by the Editorial Board for suitability of publication within the scope of the Journal and for manuscript preparation in line with the Journal guideline. Successful manuscripts are then sent to a minimum of two independent assessors for peer review in a "double blind" manner. Two assessors' reports must agree for the Board to make a decision concerning acceptance or rejection of a manuscript, however the final decision on any manuscript is the responsibility of the Editor-in-Chief. The review process takes between 4 to 6 weeks for completion but there is room for expedited review of any perceived ground-breaking manuscript.

Article Processing Charge

African Journal of Clinical and Experimental Microbiology is open access; therefore, authors are charged based on number of print pages (double column) of their published articles (not number of pages of their submitted manuscripts). The charge per print page is £10 (UK), €12 (Europe), $15 (US/Canada) and ₦ 5000 (Nigeria). An estimation of page charges will be mailed to the author(s) after acceptance of article for publication.

Waiver on Article Processing Charge

Authors based in some countries may enjoy some percentage waiver on article processing charge for their accepted manuscripts. Waivers are not automatic but given at the discretion of the Editorial Board and according to the World Bank Atlas classification of countries based on Gross National Income (GNI) per capita (https://data.worldbank.org/country). The following percentage waiver may be given; High-Income-Countries – 0% waiver; Upper-Middle-Income-Countries – 0% waiver; Lower-Middle-Income-Countries – up to 25% waiver; Low-Income-Countries – up to 40% waiver. Authors from countries entitled to waiver should request for this at the time of manuscript submission, and not after manuscript acceptance.

ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

Aims and scope

African Journal of Clinical and Experimental Microbiology publishes original research, review papers, case reports/series, short communications and letters to the editors, in all aspects of Medical Microbiology including Bacteriology, Virology, Rickettsiology and Chlamydiology, Mycology, Mycobacteriology and Actinomycetes, Parasitology, Molecular Genetics in relation to microorganisms and humans, Clinical Microbiology, Clinical Veterinary Microbiology, and Public Health Microbiology