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Review Article **Open Access**

A narrative review exploring phage therapy as a sustainable alternative solution to combat antimicrobial resistance in Africa: Applications, challenges and future directions

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Abstract:

The increasing threat of antimicrobial resistance (AMR) in Africa, coupled with limited access to advanced antibiotics and high rates of bacterial infections poses serious public health challenge. Bacteriophages, viruses that target and destroy bacteria, present a promising alternative or complementary therapy to traditional antibiotics. Phage therapy leverages its unique ability to target specific bacterial strains without affecting the host beneficial microbiota. It is an effective tool against multi-drug-resistant (MDR) microbial pathogens, particularly in resource-limited settings. This narrative review explores the potentials of phage therapy in Africa, highlighting its advantages, such as specificity, minimal side effects, and cost-effectiveness, alongside its capability to tackle biofilm-associated and AMR infections. It discusses current research and collaborations, including case studies from Nigeria, Benin, and South Africa that demonstrate the efficacy of phage therapy against bacterial pathogens such as *Escherichia coli, Acinetobacter baumannii* and *Klebsiella pneumoniae*. Furthermore, it discusses the challenges to phage therapy implementation such as regulatory hurdles, public skepticism, and infrastructure limitations, while emphasizing the importance of developing local production and awareness campaigns. The review concludes by recommending the integration of phage therapy into Africa healthcare strategies to address AMR. Through strategic partnerships, education and regulatory frameworks, phage therapy could become a transformative solution, particularly for neglected diseases and infections common in low-resource settings. As Africa seeks innovative approaches to its growing AMR crisis, phage therapy stands out as a viable and adaptable option.

Keywords: Bacteriophage; therapy; application; antimicrobial resistance; review; Africa

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Une revue narrative explorant la phagothérapie comme solution alternative durable pour lutter contre la résistance aux antimicrobiens en Afrique: applications, défis et orientations futures

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Résumé:

La menace croissante de la résistance aux antimicrobiens (RAM) en Afrique, associée à un accès limité aux antibiotiques avancés et à des taux élevés d'infections bactériennes, pose un sérieux problème de santé publique. Les bactériophages, des virus qui ciblent et détruisent les bactéries, présentent une alternative prometteuse ou une thérapie complémentaire aux antibiotiques traditionnels. La phagothérapie exploite sa capacité unique à cibler des souches bactériennes spécifiques sans affecter le microbiote bénéfique de l'hôte. C'est un outil efficace contre les agents pathogènes microbiens multirésistants (MDR), en particulier dans les environnements aux ressources limitées. Cette revue narrative explore le potentiel de la phagothérapie en Afrique, en soulignant ses avantages, tels que la spécificité, les effets secondaires minimes et la rentabilité, ainsi que sa capacité à lutter

contre les infections associées aux biofilms et à la RAM. Elle aborde les recherches et collaborations actuelles, notamment les études de cas du Nigéria, du Bénin et de l'Afrique du Sud qui démontrent l'efficacité de la phagothérapie contre des agents pathogènes bactériens tels qu'*Escherichia coli, Acinetobacter baumannii* et *Klebsiella pneumoniae*. En outre, elle aborde les défis liés à la mise en œuvre de la phagothérapie, tels que les obstacles réglementaires, le scepticisme du public et les limitations des infrastructures, tout en soulignant l'importance de développer une production locale et des campagnes de sensibilisation. La revue conclut en recommandant l'intégration de la phagothérapie dans les stratégies de santé en Afrique pour lutter contre la RAM. Grâce à des partenariats stratégiques, à l'éducation et aux cadres réglementaires, la phagothérapie pourrait devenir une solution transformatrice, en particulier pour les maladies négligées et les infections courantes dans les milieux à faibles ressources. Alors que l'Afrique cherche des approches innovantes pour faire face à la crise croissante de la RAM, la phagothérapie apparaît comme une option viable et adaptable.

Mots-clés: Bactériophage; thérapie; application; résistance aux antimicrobiens; revue; Afrique

Introduction:

Bacteriophages, often referred to as phages, are viruses that target and replicate inside bacterial cells. These viruses are incredibly abundant and diverse, inhabiting environments rich in bacterial life, including soil, water, and the human microbiome. Each type of bacteriophage typically has a high degree of specificity, often infecting a specific species of bacteria, which allows them to precisely target harmful bacteria without affecting beneficial microbiota (1,2). Phages combat bacterial infections by attaching to bacteria, injecting their genetic material, and hijacking the bacteria machinery to replicate. The process ultimately results in cell lysis, releasing new phage particles to infect other bacteria. This selective bacterial killing process is particularly valuable in treating infections, as phages reduce the bacterial population while sparing the surrounding non-targeted bacteria (3,4).

Phage therapy, a treatment that uses viruses to combat bacterial infections, was initially investigated in the early 20th century. However, with the advent and widespread use of antibiotics, it was largely abandoned. This concept capitalizes on the natural predator-prey relationship between phages and bacteria. After its discovery, phage therapy showed early promise as an effective method for treating bacterial infections particularly in Eastern Europe (5,6). The recent surge in antibiotic-resistant bacteria has led to a renewed interest in phage therapy as a potential alternative or complementary approach to conventional antibiotic treatments. Unlike broad-spectrum antibiotics, phages can penetrate biofilms, which are dense bacterial communities that protect bacteria from many conventional treatments. This characteristic makes them especially effective against biofilm-associated infections, which are often resistant to traditional antibiotics (7).

Current studies highlight phages adaptability in addressing multi-drug-resistant (MDR) bacterial infections, which are becoming increasingly common worldwide. This adaptability, along with the ability to evolve alongside bacterial resistance mechanisms, places phages as a valuable tool in combating antibiotic resistance, especially in regions where resources and access to advanced antibiotics are limited (8,9).

The resurgence of phage therapy is closely tied to the global rise in antibiotic-resistant infections, which pose a major threat to public health. By 2050, it is estimated that antibiotic-resistant infections could cause up to 10 million deaths annually if left unchecked (10). Phage therapy has demonstrated pot- ential in addressing MDR bacterial strains and is increasingly recognized as a viable complement to traditional antibiotics. Advances in phage research have led to the development of engineered phages and phage cocktails capable of targeting broader spectrum of bacterial pathogens, further increasing the feasibility of phage therapy in clinical settings (8,11).

Africa faces a growing burden of AMR infections, exacerbated by limited healthcare resources, high infection rates, and restricted access to advanced antibiotics. In regions where sanitation and healthcare infrastructure are underdeveloped, the incidence of bacterial infections and their antibiotic-resistant forms is high, contributing to significant morbidity and mortality (12,13,14). The World Health Organization (WHO) has highlighted antibiotic resistance as a critical health concern in Africa, particularly with resistant strains of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* increasingly detected (15,16).

Phage therapy offers an innovative solution to the AMR crisis in Africa by providing a targeted, adaptable, and cost-effective alternative for treating bacterial infections. Since bacteriophages can be isolated from local environments and even tailored to specific bacterial strains, phage therapy could provide a viable and accessible treatment for infections in African countries (17). In recent years, international and local initiatives have sought to develop phage therapy in Africa to address antibiotic-resistant pathogens. With collaborations between African research institutions and international bodies, phage research and application in the African healthcare system could become an effective approach to curbing resistant infections and reducing the reliance on imported antibiotics (18).

Antimicrobial resistance in Africa: A growing threat

Antibiotic resistance is a major public health issue in Africa, with rising resistance rates among many bacterial pathogens. Treatment for these infections is particularly difficult in resource-limited settings due to limited access to advanced antibiotics and alternative therapies. The major MDR pathogens in Africa, such as *E. coli*, *K. pneumoniae*, *S. aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* often cause severe infections like urinary tract infections (UTIs), bloodstream infections, respiratory and wound infections. *Escherichia coli* and *K. pneumoniae*, which are also commonly implicated in UTIs and bloodstream infections, have currently demonstrated increased resistance to crucial antibiotics such as fluoroquinolones and third-generation cephalosporins due to production of extended spectrum β-lactamase (ESBL) enzyme. *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA) are prevalent in both community and hospital settings, causing skin and soft tissue infections and worsening wound infections (12,13,19).

According to the World Health Organization (WHO), African region faces a particularly high burden of AMR, which has worsened due to inadequate surveillance systems, lack of diagnostic capabilities, and poor infection prevention and control practices. Reports from various African countries indicate that resistant strains are widespread and that MDR bacterial infections have increased mortality rates and prolonged hospital stays, straining already limited healthcare resources (16,20).

Limited healthcare infrastructure contributes to rising AMR in Africa:

Africa faces numerous healthcare challenges that contribute to the rising antibiotic resistance rates across the continent. One major issue is the limited healthcare infrastructure in many African countries, with shortages of healthcare facilities, trained medical personnel, and diagnostic equipment. Without adequate diagnostic capabilities, infections are often empirically treated with antibiotics, sometimes inappropriately, contributing to the development and spread of resistance (21-23). Self-medication is also prevalent in many parts of Africa due to easy accessibility to antibiotics without prescriptions. Many people buy antibiotics over-the-counter at pharmacies or from informal vendors, often using them improperly, such as by taking sub-therapeutic doses or using them to treat viral infections. This misuse accelerates the development of resistance (24,25).

Furthermore, poor regulation and oversight of antibiotic sales contribute significantly to resistance. In some countries, weak regulatory frameworks allow for unregulated distribution and sale of antibiotics, including counterfeit or substandard drugs that may have reduced efficacy. This lack of regulation complicates efforts to control antibiotic use, as patients can easily access antibiotics without medical authorization, further fueling misuse and resistance (26,27). Additionally, limited access to clean water, poor sanitation, and overcrowded hospitals facilitate the transmission of infections, particularly in low-resource settings, making it difficult to contain outbreaks of antibiotic-resistant bacteria (28).

Public health implications: Threat of AMR and urgent need for alternative treatment options:

The escalating crisis of antibiotic resistance in Africa poses a significant threat to public health. The increasing prevalence of drug-resistant bacteria, coupled with limited access to effective treatments in resource-constrained settings, makes these infections increasingly difficult to manage. The prevalence of MDR infections often results in extended hospital stays, limited treatment options, and higher healthcare expenses due to the need for last-resort antibiotics or alternative treatments. These impacts strain already limited healthcare resources in many African countries (29-31).

One of the most significant public health impacts of MDR bacteria is the threat they pose to vulnerable populations, such as neonates, immunocompromised patients, and individuals with chronic illnesses, thus, contributing to a high burden of preventable mortality in Africa, where limited access to advanced treatments exacerbates the impact of resistant infections (13,32,33). The urgent need for alternative therapies is underscored by the slow development of new antibiotics and the diminishing effectiveness of existing drugs.

Bacteriophage therapy, for instance, is emerging as a promising alternative for treating MDR infections by using viruses that specifically target and destroy pathogenic bacteria. Phage therapy and other alternatives like antimicrobial peptides and probiotics could offer valuable options to supplement or replace antibiotics, especially as the continent grapples with the severe consequences of antibiotic resistance (34,35).

The promise of phage therapy in tackling AMR in Africa:

As antimicrobial resistance continues to challenge healthcare systems globally, phage therapy presents an alternative and promising solution, particularly in Africa, where access to effective antibiotics is limited and AMR is widespread. Phage therapy unique benefits, such as its specificity to pathogens, minimal side effects, and effectiveness against drug-

resistant bacteria, make it a strong candidate for tackling AMR on the continent. In addition to clinical effectiveness, phage therapy also offers cost-effective production and ease of storage, making it a practical and accessible choice for resource-limited regions (6,8).

Advantages of phage therapy over antibiotics:

One primary benefit is its specificity to pathogens, which allows for targeted treatment without harming the host beneficial microbiota. Unlike broad-spectrum antibiotics, bacteriophages (phages) selectively infect and kill specific bacteria, reducing the risk of disrupting the natural microbial balance and decreasing the likelihood of secondary infections or adverse reactions (36,37).

Moreover, phage therapy is particularly effective against MDR bacteria, providing a viable treatment option where antibiotics have failed. This feature is essential in Africa, where AMR levels are high and last-resort antibiotics are scarce. Phages can also evolve alongside bacterial pathogens, potentially reducing the risk of resistance development and prolonging the efficacy of treatment (38,39).

Phage therapy generally has fewer side effects than antibiotics, as it is less likely to trigger allergic reactions or gastrointestinal disturbances commonly associated with antibiotic use. Additionally, because phages are natural components of the environment, they are usually well-tolerated by the human body, further enhancing their safety profile (34,40).

Suitability of phage therapy in the African context:

In Africa, where infections caused by drug-resistant pathogens are increasingly common, phage therapy offers a tailored solution to combating diseases prevalent on the continent. Pathogens such as *S. aureus*, *K. pneumoniae*, and those responsible for diarrhoeal diseases are common targets of phage therapy. For instance, *S. aureus*, including MRSA strain, is a frequent cause of wound and soft tissue infections. Phage therapy has demonstrated efficacy against MRSA in various settings, suggesting it could be valuable in Africa, where resistant infections are particularly problematic in hospital and community environments (41,42).

Klebsiella pneumoniae, known for causing severe respiratory and urinary tract infections, is another target for phage therapy. This pathogen is often resistant to multiple antibiotics, especially in African healthcare settings where treatment options are limited. Phages targeting *Klebsiella* strains have shown promise in experimental studies, providing a potential alternative treatment for these infections. Additionally, phage therapy could be adapted for use against pathogens causing diarrhoeal diseases, which are significant contributors to morbidity and mortality among children in Africa. Phages have been successfully used to treat *E. coli* and *Shigella* species, both major causes of bacterial diarrhoea on the continent, highlighting their potential to reduce the disease burden (43,44,45).

Cost and accessibility of phage therapy:

Phage therapy has the potential to be cost-effective and accessible, especially when compared to the high development and production costs associated with new antibiotics. Phages can be isolated from natural sources and adapted for therapeutic use with relatively low investment in laboratory infrastructure. Local production of phage preparations is feasible in many African countries, particularly with minimal equipment requirements, as opposed to the large-scale industrial facilities needed for conventional antibiotics. This adaptability is significant for low-resource settings in Africa, where healthcare budgets are often constrained (46,47).

Another advantage is the ease of storage and distribution. Phages are stable at a wide range of temperatures and can often be stored without refrigeration, making them suitable for regions where cold-chain storage is difficult. This storage advantage reduces logistical barriers and makes phage therapy especially viable in rural or remote areas with limited access to healthcare facilities. With these benefits, phage therapy could be implemented at a lower cost and with greater accessibility, providing a valuable alternative to traditional antibiotic treatments (48,49).

Current research and applications of phage therapy in Africa

Research into phage therapy has been gaining momentum in Africa as the continent grapples with high rates of antibiotic resistance. Various institutions and partnerships are involved in investigating phage therapy effectiveness, adaptability, and potential for implementation within African healthcare systems. This research spans experimental studies, clinical trials, and international collaborations aimed at exploring phage therapy as a sustainable solution to the growing crisis of AMR on the continent

Case study in Ethiopia:

A study in Ethiopia showed that ΦJHS and ΦSMK phages demonstrated broad lytic spectra on clinical MDR *P. aeruginosa* strains, as all strains tested were lysed by both phages. Seven MDR *P. aeruginosa* strains were infected, suggesting that the isolated phages might be used as biocontrol agents on abiotic surfaces or as candidates for clinical phage therapy.

Additionally, it may be possible to create

a phage cocktail using the isolated phages to target a specific type of bacterium. Such a cocktail that targets only a single rather than multiple bacterial species is described as generally emphasizing the spectrum of phage activity breadth in its design, rather than necessarily emphasizing the spectrum of phage activity depth. The promising outcomes of phages being used for both bacterial colonization prevention and bacterial biofilm removal are highlighted by the reduction of MDR *P. aeruginosa* biofilm development on catheter and endotracheal tube surfaces (50).

Lytic phages against *Escherichia coli* and *Pseudomonas aeruginosa* in Nigeria:

Another case study in Nigeria explored the use of phages to treat *E. coli* and *P. aeruginosa*. Clinical isolates resistant to multiple antibiotics were treated with phage cocktails, leading to the successful eradication of the pathogens. The phages recovered from wastewaters were exclusive *to E. coli* and MDR *P. aeruginosa*. These demonstrate the value of using phages as therapeutic agents to lessen the threat posed by AMR pathogens (51,52).

Joint initiatives in Benin:

From hospital wastewater in Benin, scientists discovered and described three new *A. baumannii* phages that belong to the Autographiviridae family. The opportunistic bacterium, *A. baumannii* is primarily linked to infections acquired in hospitals. The isolated phages satisfy the specifications needed to be employed in phage therapy. Their limited host range, however, may restrict their potential for therapeutic use (53).

Compassionate use in South Africa:

The South African healthcare settings have implemented phage therapy under compassionate use programs. In a study in South Africa, a significant log reduction in viable *E. coli* O177 cell counts was observed on beef samples upon phage treatment over the 7-day incubation period. *E. coli* cell counts were lowered by two single phages and three phage cocktails to values below the detection limit (1.0 log10 CFU/g).

For both individual phages and cocktails, log decrease in viable *E. coli* cell counts varied between 2.10 - 7.81 CFU/g and 2.86 - 7.81 CFU/g, respectively. Both single phage and phage cocktails prevented the growth of *E. coli* O177 biofilms, with phage cocktails demonstrating the highest level of effectiveness. Additionally, phage mixtures were more effective than single phage at eliminating pre-formed biofilm. These results led to the conclusion that phage cocktails created in this work may be utilized to decrease *E. coli* O177 infection (54).

Challenges of phage therapy implementation in Africa:

Despite the potential benefits of phage therapy, implementing it in African healthcare systems faces several challenges. These include regulatory hurdles, public and healthcare sector skepticism, and infrastructure limitations for production and storage.

Regulatory hurdles:

The lack of clear and standardized regulatory frameworks for phage therapy in most African countries presents a significant barrier to its implementation. Unlike antibiotic therapy that has well-established pathways for approval, phage therapy lacks formalized protocols and guidelines for preclinical and clinical trials in Africa. This ambiguity hampers the widespread adoption of phage-based treatments (55).

There are global inconsistencies in the regulatory frameworks for phage therapy, with fragmented regulations. While countries such as Georgia and Poland have established systems, others, including most African countries, have no established regulatory frameworks (56). Efforts by the WHO to streamline regulatory processes for novel therapies could help, but these measures are still in the early stages.

Public and healthcare sector Skepticism:

Phage therapy is often viewed with skepticism due to its novelty and historical associations with limited regions. Many doctors and healthcare providers in Africa are unfamiliar with phage therapy, leading to unwillingness and resistance to adopting it over traditional antibiotics (57). Public acceptance is low because phages are misunderstood, with fears rooted in their association with viruses and biological warfare.

Addressing these misconceptions through education campaigns and showcasing successful case studies are essential (58). Cultural factor is also a challenge. In some communities, distrust of modern medicine complicates the introduction of phage therapy, requiring culturally sensitive awareness campaigns (59).

Production and storage challenges:

The practicalities of producing and storing bacteriophages present hurdles in regions with limited resources. Producing phages requires advanced laboratory facilities to ensure safety, efficacy, and specificity (60). Few African countries have the infrastructure for large-scale, high-quality phage production. Developing formulations suitable for different infections e. g., topical, oral, or injectable demands significant investment in research and development.

Phages are temperature-sensitive and often require cold-chain storage to maintain viability (8), which is a challenge in regions with unreliable electricity or refrigeration. For instance, in rural areas of Africa, phages risk degradation due to inadequate cold storage, which undermines their effectiveness and limits their application in community healthcare settings. The cost implication of phage therapy is also a hurdle.

Establishing the necessary infrastructure for production and storage can be prohibitively expensive for many African nations, particularly those heavily reliant on international aid for healthcare needs (56,61).

Future prospects of phage therapy in Africa:

Further research is essential to realize the full potential of phage therapy in Africa. Large-scale, randomized controlled trials are needed to establish the safety, efficacy, and optimal dosing of phages. Trials focused on region-specific infections such as typhoid fever or cholera, can validate the relevance of phage therapy in African settings (1). Also, integrating phage therapy into healthcare systems requires strategic planning and partnerships. Collaborations with pharmaceutical companies can address challenges in mass production and distribution. Examples from Eastern Europe, such as the Eliava Institute, illustrate the viability of such models (8).

International collaborations such as those with WHO and the Africa CDC, aim to establish regional guidelines for phage therapy trials and usage. Incorporating phage therapy into national AMR strategies would promote its systematic use and scaling. For example, training hospital microbiologists to isolate and produce phages locally could support personalized therapy initiatives.

Conclusion:

With the continent burden of infectious diseases, limited access to new antibiotics, and increasing prevalence of AMR, phage therapy represents a transformative opportunity. Furthermore, its potential to address neglected tropical diseases and its adaptability to local healthcare needs underscore its relevance in improving public health outcomes across the region (6,62).

To fully harness the potential of phage therapy in Africa, coordinated efforts are required from policy-makers, researchers and healthcare providers. Governments must prioritize the establishment of regulatory frameworks that facilitate phage therapy development and integration into healthcare systems. Investing in infrastructure for local phage production and quality assurance is crucial. African research institutions should focus on conducting clinical trials, optimizing phage formulations, and studying phage-host interactions in the context of regional pathogens. Training and educating medical professionals about phage therapy can foster its acceptance and successful implementation in clinical settings.

By adopting phage therapy as part of a multi-pronged strategy to address AMR, Africa can strengthen its healthcare systems and provide innovative, sustainable solutions to its pressing public health challenges. The continent leadership in advancing phage therapy could also position it as a global pioneer in this promising field.

Contributions of authors:

NOO was involved in research conceptualization, design formulation and writing of the original draft; NFE was involved in research design, supervision, methodological activities, analysis, writing and review of the manuscript. All authors read and approved the manuscript for submission.

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References:

1. Abedon, S. T., García, P., Mullany, P., and Aminov, R. Editorial: Phage Therapy: Past, Present and Future. Front Microbiol. 2017; 8: 981. https://doi.org/10.3389/fmicb.2017.00981
2. Burrowes, B., Harper, D. R., Anderson, J., McConville, M., and Enright, M. C. Bacteriophage therapy: potential uses in the control of antibiotic resistant pathogens. Exp Rev Anti-infect Ther. 2011; 9 (9): 775–785. https://doi.org/10.1586/eri.11.90
3. Chan, B. K., Abedon, S. T., and Loc-Carrillo, C. Phage cocktails and the future of phage therapy. Future Microbiol. 2013; 8 (6): 769–783. https://doi.org/10.2217/fmb.13.47
4. Chang, C., Yu, X., Guo, W., et al. Bacteriophage-Mediated Control of Biofilm: A Promising New Dawn for the Future. Front Microbiol. 2022; 13: 825828. https://doi.org/10.3389/fmicb.2022.825828
5. Doss, J., Culbertson, K., Hahn, D., Camacho, J., and Barekzi, N. A Review of Phage Therapy against Bacterial Pathogens of Aquatic and Terrestrial Organisms. Viruses. 2017; 9 (3): 50. https://doi.org/10.3390/v9030050
6. Pires, D. P., Cleto, S., Sillankorva, S., Azeredo, J., and Lu, T. K. Genetically Engineered Phages: a

Review of Advances over the Last Decade. Microbiol Mol Biol Rev.2016; 80 (3): 523–543. https://doi.org/10.1128/MMBR.00069-15
7. Dufour, N., Debarbieux, L., Fromentin, M., and Ricard, J. D. Treatment of Highly Virulent Extra-intestinal Pathogenic *Escherichia coli* Pneumonia With Bacteriophages. Crit Care Med. 2015; 43 (6): e190–e198. https://doi.org/10.1097/CCM.0000000000000968
8. Kortright, K. E., Chan, B. K., Koff, J. L., and Turner, P. E. Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. Cell Host Microbe. 2019; 25 (2): 219–232. https://doi.org/10.1016/j.chom.2019.01.014
9. Rogovski, P., Cadamuro, R. D., da Silva, R., et al. Uses of Bacteriophages as Bacterial Control Tools and Environmental Safety Indicators. Front Microbiol. 2021; 12: 793135. https://doi.org/10.3389/fmicb.2021.793135
10. O'Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance. Wellcome Trust and HM Government. 2016 https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf
11. Górski, A., Międzybrodzki, R., Węgrzyn, G., et al. Phage therapy: Current status and perspectives. Med Res Rev. 2020; 40 (1): 459–463. https://doi.org/10.1002/med.21593
12. Tadesse, B. T., Ashley, E. A., Ongarello, S., et al. Antimicrobial resistance in Africa: a systematic review. BMC Infect Dis. 2017; 17 (1): 616. https://doi.org/10.1186/s12879-017-2713-1
13. Essack, S. Y., Desta, A. T., Abotsi, R. E., and Agoba, E. E. Antimicrobial resistance in the WHO African region: current status and roadmap for action. J Publ Health.2017; 39 (1): 8–13. https://doi.org/10.1093/pubmed/fdw015
14. Ekpunobi, N. F., Adesanoye, S., Orababa, et.al. Public health perspective of public abattoirs in Nigeria, challenges and solutions. GSC Biol Pharmaceut Sci. 2024; 26 (2): 115-127
15. Ekpunobi, N. F., and Isaac, A. A. Phenotypic characterization of biofilm formation and efflux pump activity in multi-drug-resistant *Staphylococcus* species isolated from asymptomatic students. J Microbiol Exp. 2020; 8: 223–229
16. World Health Organization. Antibacterial agents in clinical development: An analysis of the antibacterial clinical development pipeline, including tuberculosis. 2018
17. Monteiro, R., Pires, D. P., Costa, A. R., and Azeredo, J. Phage Therapy: Going Temperate? Trends Microbiol. 2019; 27 (4): 368–378. https://doi.org/10.1016/j.tim.2018.10.008
18. Luong, T., Salabarria, A. C., and Roach, D. R. Phage Therapy in the Resistance Era: Where Do We Stand and Where Are We Going? Clin Therap. 2020; 42 (9): 1659–1680. https://doi.org/10.1016/j.clinthera.2020.07.014
19. Newman, M. J., Frimpong, E., Donkor, E. S., Opintan, J. A., and Asamoah-Adu, A. Resistance to antimicrobial drugs in Ghana. Infection and drug resistance. 2011; 4: 215–220. https://doi.org/10.2147/IDR.S21769
20. Bunduki, G. K., Masoamphambe, E., Fox, T., Musaya, J., Musicha, P., and Feasey, N. Prevalence, risk factors, and antimicrobial resistance of endemic healthcare-associated infections in Africa: a systematic review and meta-analysis. BMC Infect Dis. 2024; 24 (1): 158. https://doi.org/10.1186/s12879-024-09038-0
21. Ekpunobi, N., Markjonathan, I., Olanrewaju, O., and Olanihun, D. Idiosyncrasies of COVID-19; A Review. Iran J Med Microbiol. 2020; 14: 290–296.
22. Bebell, L. M., and Muiru, A. N. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? Global Heart. 2014; 9 (3): 347–358. https://doi.org/10.1016/j.gheart.2014.08.009
23. Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., et al. Antimicrobial resistance in developing countries Part I: recent trends and current status. Lancet Infect Dis. 2005; 5 (8): 481–493 https://doi.org/10.1016/S1473-3099(05)70189-4
24. Ocan, M., Obuku, E. A., Bwanga, F., et al. Household antimicrobial self-medication: a systematic review and meta-analysis of the burden, risk factors and outcomes in developing countries. BMC Publ Health. 2015; 15: 742. https://doi.org/10.1186/s12889-015-2109-3
25. Aslam, A., Gajdács, M., Zin, C. S., et al. Evidence of the Practice of Self-Medication with Antibiotics among the Lay Public in Low- and Middle-Income Countries: A Scoping Review. Antibiotics (Basel, Switzerland), 2020; 9 (9): 597. https://doi.org/10.3390/antibiotics9090597
26. Mendelson, M., and Matsoso, M. P. The World Health Organization Global Action Plan for Antimicrobial Resistance. 2015; 105 (5): 325. https://doi.org/10.7196/samj.9644
27. Founou, R. C., Founou, L. L., and Essack, S. Y. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One. 2017; 12 (12): e0189621. https://doi.org/10.1371/journal.pone.0189621
28. Tessema, G. A., Kinfu, Y., Dachew, B. A., et al. The COVID-19 pandemic and healthcare systems in Africa: a scoping review of preparedness, impact and response. BMJ Glob Health, 2021; 6 (12): e007179. https://doi.org/10.1136/bmjgh-2021-007179
29. Ayukekbong, J. A., Ntemgwa, M., and Atabe, A. N. The threat of antimicrobial resistance in developing countries: causes and control strategies Antimicrob Resist Infect Contr. 2017; 6: 47. https://doi.org/10.1186/s13756-017-0208-x
30. Kakkar, A. K., Shafiq, N., Singh, G., et al. Antimicrobial Stewardship Programs in Resource-Constrained Environments: Understanding and Addressing the Need of the Systems. Front Publ Health. 2020; 8: 140. https://doi.org/10.3389/fpubh.2020.00140
31. Enyi, E., and Ekpunobi, N. Secondary metabolites from endophytic fungi of *Moringa oleifera*: Antimicrobial and antioxidant properties. J Microbiol Exp. 2022; 10: 150-154. http://doi.org/10.15406/jmen.2022.10.00367
32. Reardon, S. Antibiotic resistance sweeping the developing world. Nature, 2014; 509 (7499): 141–142.https://doi.org/10.1038/509141a
33. Ekpunobi, N. F., Mgbedo, U. G., Okoye, L. C., and Agu, K. C. Prevalence of ESBL genes in *Klebsiella pneumoniae* from individuals with community-acquired urinary tract infections in Lagos state, Nigeria. J RNA Genomics. 2024; 20 (2): 1-6.
34. Aslam, B., Wang, W., Arshad, M. I., et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018; 11: 1645 – 1658. https://doi.org/10.2147/IDR.S173867
35. Helmy, Y. A., Taha-Abdelaziz, K., Hawwas, H. A. E., et al. Antimicrobial Resistance and Recent Alternatives to Antibiotics for the Control of Bacterial Pathogens with an Emphasis on Food-borne Pathogens. Antibiotics (Basel, Switzerland). 2023; 12 (2): 274. https://doi.org/10.3390/antibiotics12020274
36. Sulakvelidze, A., Alavidze, Z., and Morris, J. G., Jr. Bacteriophage therapy. Antimicrob Agents Chemother. 2001; 45 (3): 649–659. https://doi.org/10.1128/AAC.45.3.649-659.2001
37. Nobrega, F. L., Costa, A. R., Kluskens, L. D., and Azeredo, J. Revisiting phage therapy: new applications for old resources. Trends Microbiol. 2015; 23 (4): 185–191. https://doi.org/10.1016/j.tim.2015.01.006
38. Pirnay, J. P., Verbeken, G., Ceyssens, P. J., et al. The Magistral Phage. Viruses. 2018; 10 (2): 64. https://doi.org/10.3390/v10020064
39. Kakasis, A., and Panitsa, G. Bacteriophage therap as an alternative treatment for human infections. A comprehensive review. Int J Antimicrob Agents. 2019; 53 (1): 16–21.

https://doi.org/10.1016/j.ijantimicag.2018.09.004

40. Górski, A., Międzybrodzki, R., Łobocka, M., et al. Phage Therapy: What Have We Learned? Viruses. 2018; 10 (6): 288. https://doi.org/10.3390/v10060288
41. Bernabé, K. J., Langendorf, C., Ford, N., Ronat, J. B., and Murphy, R. A. Antimicrobial resistance in West Africa: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017; 50 (5): 629–639. https://doi.org/10.1016/j.ijantimicag.2017.07.002
42. Iseppi, R., Mariani, M., Condò, C., Sabia, C., and Messi, P. Essential Oils: A Natural Weapon against Antibiotic-Resistant Bacteria Responsible for Nosocomial Infections. Antibiotics (Basel, Switzerland). 2021; 10 (4): 417. https://doi.org/10.3390/antibiotics10040417
43. Caflisch, K. M., Suh, G. A., and Patel, R. Biological challenges of phage therapy and proposed solutions: a literature review. Exp Rev Anti-infect Ther 2019; 17 (12): 1011–1041. https://doi.org/10.1080/14787210.2019.1694905
44. Melo, L. D. R., Pinto, G., Oliveira, F., et al. The Protective Effect of *Staphylococcus epidermidis* Biofilm Matrix against Phage Predation. Viruses. 2020; 12 (10): 1076. https://doi.org/10.3390/v12101076
45. Ekpunobi, N. F., Mgbedo, U. G., Okoye, L. C., and Agu, K. C. Prevalence of ESBL Genes in *Klebsiella pneumoniae* from Individuals with Community-Acquired Urinary Tract Infections in Lagos State, Nigeria. Preprints. 2024. https://doi.org/10.20944/preprints202403.1702.v1
46. Salmond, G. P., and Fineran, P. C. A century of the phage: past, present and future. Nature reviews. Microbiol. 2015; 13 (12): 777–786. https://doi.org/10.1038/nrmicro3564
47. Manohar, P., Tamhankar, A. J., Lundborg, C. S., and Nachimuthu, R. Therapeutic Characterization and Efficacy of Bacteriophage Cocktails Infecting *Escherichia coli, Klebsiella pneumonia*e, and *Enterobacter* species. Front Microbiol. 2019; 10: 574. https://doi.org/10.3389/fmicb.2019.00574
48. Międzybrodzki, R., Borysowski, J., Weber-Dąbrowska, B., et al. Clinical aspects of phage therapy. Adv Virus Res. 2012; 83: 73–121. https://doi.org/10.1016/B978-0-12-394438-2.00003-7
49. Anastassopoulou, C., Ferous, S., Petsimeri, A., Gioula, G., and Tsakris, A. Phage-Based Therapy in Combination with Antibiotics: A Promising Alternative against Multidrug-Resistant Gram-Negative Pathogens. Pathogens (Basel, Switzerland). 2024; 13 (10): 896. https://doi.org/10.3390/pathogens13100896
50. Amankwah, S., Adisu, M., Gorems, K., Abdella, K., and Kassa, T. Assessment of Phage-Mediated Inhibition and Removal of Multidrug-Resistant *Pseudomonas aeruginosa* Biofilm on Medical Implants. Infect Drug Resist. 2022; 15: 2797-2811.https://doi.org/10.2147/IDR.S367460
51. Emencheta, S. C., Eze, C. C., Attama, A. A., Agbo, D. E., and Onuigbo, E. B. Isolation of *Pseudomonas aeruginosa* phages from wastewaters. Hosts and Viruses. 2021; 8 (6): 1-6.
52. Emencheta, S. C., Attama, A.A., Ezeibe, E. N., Agubata, A. A., and Onuigbo, E. B. Isolation of *Escherichia coli* phages from wastewaters. Hosts and Viruses. 2022: 9: 12-18.
53. Kolsi, A., Haukka, K., Dougnon, V. *et al.* Isolation and characterization of three novel *Acinetobacter baumannii* phages from Beninese hospital wastewater. Arch Virol. 2023; 168: 228. https://doi.org/10.1007/s00705-023-05845-z
54. Montso, P. K., Mlambo, V., and Ateba, C. N. Efficacy of novel phages for control of multi-drug resistant *Escherichia coli* O177 on artificially contaminated beef and their potential to disrupt biofilm formation. Food Microbiol. 2021; 94*:* 103647. https://doi.org/10.1016/j.fm.2020.103647
55. Henein, A. What are the limitations on the wider therapeutic use of phage? Bacteriophage. 2013; 3 (2): e24872. https://doi.org/10.4161/bact.24872
56. Fabiyi, K., Sintondji, K., Agbankpe, J., Assogba, P., Koudokpon, H., Lègba, B., Gbotche, E., Baba-Moussa, L., and Dougnon, V. Harnessing Bacterio phages to Combat Antibiotic-Resistant Infections in Africa: A Comprehensive Review. Antibiotics (Basel, Switzerland). 2024; 13 (9): 795. https://doi.org/10.3390/antibiotics13090795
57. Pires, D. P., Costa, A. R., Pinto, G., Meneses, L., and Azeredo, J. Current challenges and future opportunities of phage therapy. FEMS microbiol Rev. 2020; 44 (6): 684–700. https://doi.org/10.1093/femsre/fuaa017
58. Anomaly, J. The Future of Phage: Ethical Challenges of Using Phage Therapy to Treat Bacterial Infections. Publ Health Ethics. 2020; 13 (1): 82–88.https://doi.org/10.1093/phe/phaa003
59. Hamer, D. H., Herlihy, J. M., Musokotwane, K., et al. Engagement of the community, traditional leaders, and public health system in the design and implementation of a large community-based, cluster-randomized trial of umbilical cord care in Zambia. Am J Trop Med Hyg. 2015; 92 (3): 666–672. https://doi.org/10.4269/ajtmh.140218
60. Kutateladze, M., and Adamia, R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol. 2010; 28 (12): 591–595. https://doi.org/10.1016/j.tibtech.2010.08.001
61. Wenham, C., Wouters, O., Jones, C., et al. Measuring health science research and development in Africa: mapping the available data. Health Res Policy syst. 2021; 19 (1): 142. https://doi.org/10.1186/s12961-021-00778-y
62. Ekpunobi, N. F., and Agu, K. C. Emergence and Re-Emergence of Arboviruses: When Old Enemies Rise Again. Cohesive J Microbiol Infect Dis. 2024; 7 (2): 000658. doi:10.31031/CJMI.2024.07.000658.

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Project Report **Open Access**

The journey to institutionalising Antimicrobial Stewardship (AMS) in a resource-constrained tertiary healthcare facility in Lagos, Nigeria

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Abstract:

Background: Antimicrobial stewardship (AMS) is one of the main strategies to stem the global tide of antimicrobial resistance (AMR). While developed nations have successfully implemented antimicrobial stewardship programmes, such initiatives remain underdeveloped in many Nigerian healthcare institutions. This is a report of a project to improve the AMS programme at the Lagos University Teaching Hospital (LUTH), Nigeria, by strengthening the antimicrobial stewardship committee (AMSC) and antimicrobial stewardship team (AMST) of the hospital.
Methodology: The Commonwealth Partnerships for Antimicrobial Stewardship (CwPAMS) facility assessment tool was used to identify gaps in the AMS programme (ASP) of LUTH, and activities to address the gaps were conducted over a 3-year period (2021-2024).
Results: The results of the activities to address the identified gaps in the ASP were the expansion of AMS activities from 3 to 10 departments of the hospital, a strong management commitment and support, consistent antibiotic consumption calculations, strategic training of professionals, and increased knowledge and awareness of AMS among staff and students. However, major challenges identified included shortage of staff and lack of functional electronic medical records.
Conclusion: Continuing pre- and in-service training of staff, AMS activities and monitoring, and incorporation of AMS actions and interventions performed with the electronic medical records are recommended for sustaining AMS in the hospital.

Keywords: AMS programme, gaps, activities, interventions, institionalise, resource-constrained

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Le cheminement vers l'institutionnalisation de la gestion des antimicrobiens (GAM) dans un établissement de soins de santé tertiaires aux ressources limitées à Lagos, au Nigéria

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Résumé:

Contexte: La gestion des antimicrobiens (GAM) est l'une des principales stratégies pour endiguer la vague mondiale de résistance aux antimicrobiens (RAM). Si les pays développés ont mis en œuvre avec succès des programmes de gestion des antimicrobiens, ces initiatives restent peu développées dans de nombreux établissements de santé Nigérians. Ce rapport présente un projet visant à améliorer le programme de GAM au Centre hospitalier universitaire de Lagos (LUTH), au Nigéria, en renforçant le comité de gestion des antimicrobiens (CMGA) et l'équipe de gestion des antimicrobiens (EMGA) de l'hôpital.
Méthodologie: L'outil d'évaluation des installations du Commonwealth Partnerships for Antimicrobial Stewardship (CwPAMS) a été utilisé pour identifier les lacunes du programme de gestion des antimicrobiens (ASP) du LUTH. Des activités visant à combler ces lacunes ont été menées sur une période de trois ans (2021-2024).
Résultats: Les activités visant à combler les lacunes identifiées dans le ASP ont permis d'étendre les activités de gestion des antimicrobiens de trois à dix services de l'hôpital, d'obtenir un engagement et un soutien solides de la direction, de calculer la consommation d'antibiotiques de manière cohérente, de former stratégiquement les professionnels et de renforcer les connaissances et la sensibilisation au bon usage des antimicrobiens parmi le personnel et les étudiants. Cependant, les principaux défis identifiés comprenaient la pénurie de personnel et l'absence de dossiers médicaux électroniques fonctionnels.
Conclusion: La poursuite de la formation initiale et continue du personnel, la poursuite des activités et du suivi de gestion des antimicrobiens, ainsi que l'intégration des actions et interventions de gestion des antimicrobiens réalisées avec les dossiers médicaux électroniques, sont recommandées pour pérenniser le bon usage des antimicrobiens à l'hôpital.

Mots-clés: Programme de gestion des antimicrobiens, lacunes, activités, interventions, institutionnalisation, contraintes de ressources

Introduction:

Antimicrobial stewardship (AMS) is an essential tool used to tackle antimicrobial resistance (1). In high-resource settings, antimicrobial stewardship programmes (ASP) have improved rational antibiotic use (2,3). Developing countries have an increased burden of infectious diseases, thus will face a greater impact of antimicrobial resistance (AMR) (3, 4). Inappropriate antibiotic use and an increase in AMR prevalence have been reported in Nigeria (5-8). Therefore, ASP in the country need strengthening.

Stewardship programmes are limited across the country. This is evidenced by a study conducted across tertiary hospitals in Nigeria, which found that just 30% had antimicrobial stewardship committees, and only 35% performed periodic Global-Point Prevalence Survey (G-PPS) to monitor and evaluate antimicrobial use (8). Another study also found that only 35% of tertiary hospitals in the country had formal stewardship programmes (9).

In 2021, only 20% of healthcare facilities in Lagos, Nigeria, had established stewardship programmes, and out of these facilities, only 24.0% performed routine antimicrobial pre-authorization while 8% performed prospective audit, intervention and feedback (PAIF) for specific antibiotics used (10). These findings emphasise the need to establish ASPs in facilities where they are non-existent, and strengthen them where they are already in place.

The Antimicrobial Stewardship Committee (AMSC) of Lagos University Teaching Hospital (LUTH) was inaugurated in 2013. As at 2016, ours was one of the few hospitals in Nigeria with a functional AMS committee, with our medical microbiology laboratory being a sentinel site for the AMR surveillance of the country. By this time, progress made included conduct of a baseline Global-point prevalence survey (G-PPS) of antimicrobial use and resistance in 2015, after which follow-up PP surveys were performed in 2017.

Raising awareness and AMS interventions in some departments improved antibio-

tic prescribing practices and by 2017, led to the reduction of antibiotic use prevalence from 82.5% to 51.1% in the hospital (11). By 2019, when our ASP was rolled out, the Antimicrobial Stewardship Teams (AMSTs) of some departments met irregularly, and teams in two departments performed prospective audit with intervention and feedback as a stewardship strategy (11). Although, this progress was encouraging, a lot still had to be done to strengthen the programme (Fig 1). There was the need to institutionalise the ASP by extending AMS interventions to all departments and bring all stakeholders on board. Hence, we were open to opportunities available to us to achieve this.

To sustain the national AMS programme, individual hospitals must have strong and functional ASPs. This can be achieved by identifying the existing gaps and developing a plan to address them. Therefore, this project sought to identify and address the gaps in the ASP in our hospital. The project aimed to improve AMS in LUTH by strengthening the stewardship committee and teams and to build the capacity of the healthcare professionals for AMS surveillance and activities.

The specific objectives were to; (i) identify gaps in the AMS activities and proffer solutions, (ii) train the stewardship committee and teams on AMS actions and interventions, (iii) train the healthcare professionals on AMS principles and practices, and (iv) sustain AMS activities.

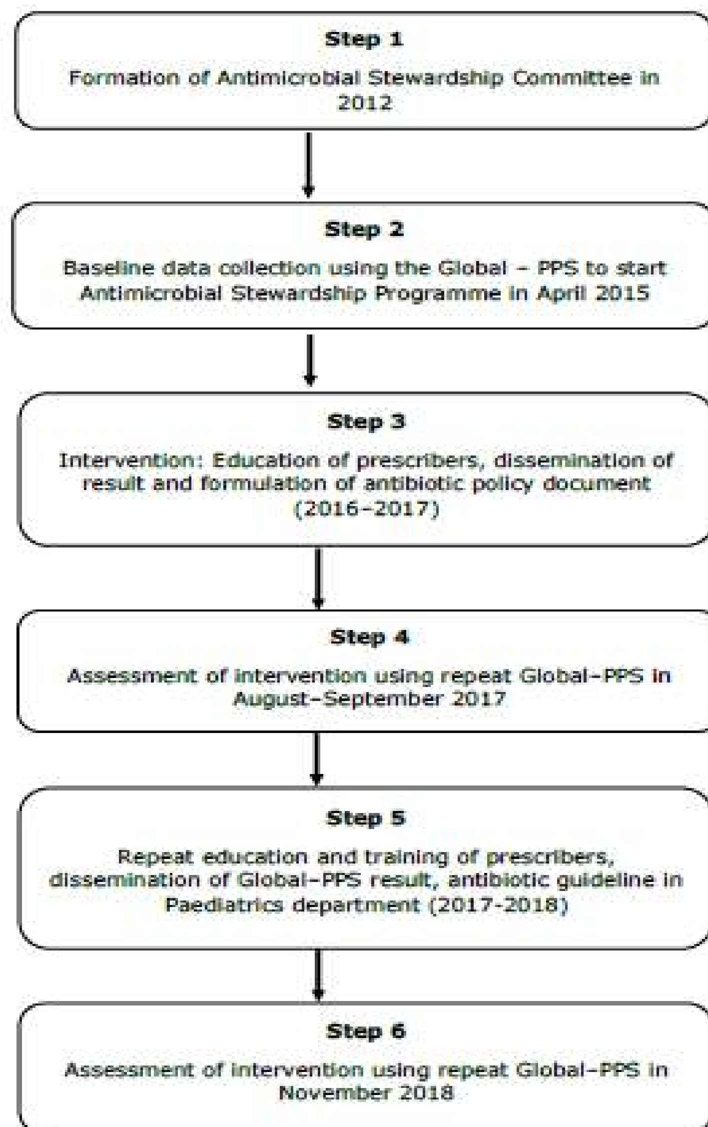

Fig 1: Steps for implementation of Antimicrobial Stewardship Programme at the Lagos University Teaching Hospital, Nigeria in 2018 (11)

Materials and method:

Study setting:

The setting is the Lagos University Teaching Hospital (LUTH), which is one of the largest tertiary hospitals in Nigeria. It has a well-equipped and functional medical microbiology laboratory, which is also a sentinel site for the national AMR surveillance. The hospital is one of the few facilities with an ASP and provides information for antimicrobial use (AMU) surveillance.

Methodology:

To achieve our aim and objectives, the Commonwealth Partnerships for Antimicrobial Stewardship (CwPAMS) facility assessment tool was used to identify the gaps (CwPAMS AMS Assessment Tool Oct 2022. docx), and the activities on Fig 2 and Box 1 were conducted over a 3-year period (2021-2024). Patient level data on AMU were collected before and during the project with the G-PPS tool. (https://www.global-pps.com/project/inpatient-module/).

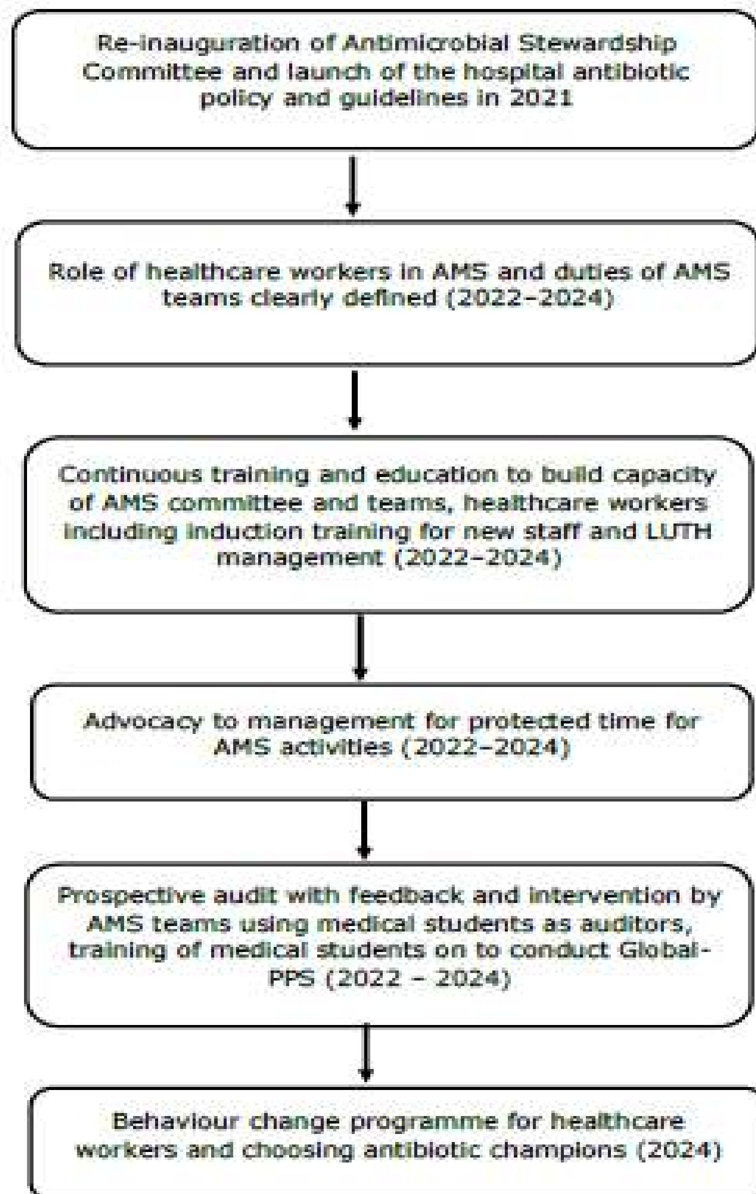

Fig 2: Flow chart of the Antimicrobial Stewardship Programme Journey in Lagos University Teaching Hospital, Nigeria (2021-2024)

Box 1: Planned activities to institutionalise ASP in Lagos University Teaching Hospital, Nigeria, in 2021

- Educational programmes to build the capacity of the AMSC, AMSTs, LUTH management and all stakeholders for AMS were done onsite.
- Training of the pharmacists on calculation of antibiotic consumption was done onsite and online.
- The roles of healthcare workers in AMSTs in the hospital wards were defined during the online and onsite trainings for nurses, pharmacists and prescribers.
- Training of medical students on prospective audit.
- Performance of audit intervention and feedback by AMSTs.
- Developing and updating of relevant policies and training documents.
- Advocacy to management to give protected time for staff AMS activities in departments was done.
- Continuation of AMS activities already in place and extension to more departments: Global-PPS, prospective audit with intervention and feedback (as a stewardship strategy), antibiotic consumption calculations by pharmacists, and raising awareness on AMS.
- Training of medical undergraduates to perform Global-PPS and pharmacist interns to perform antibiotic consumption calculations.
- Behavioural change workshop to encourage behaviour change among the healthcare workers

Results:

Identified gaps:

The major gaps identified in the ASP following assessment with the CwPAMS tool included; (i) no action plan for AMS activities and no information technology (IT) and financial support for the ASP, (ii) staff did not have training in AMS at induction or in-service level, (iii) antibiotic consumption was not being regularly calculated in the hospital, (iv) no list of restricted antibiotics, and (v) no protected time for routine AMS activities.

AMS document development/update:

Relevant documents for AMS were developed and/or updated as shown in Box 2.

Box 2: AMS documents developed/updated at Lagos University Teaching Hospital, Nigeria

- The hospital Antibiotic Policy and Guidelines document (version 1.0) was developed in 2021. It was initiated in individual departments and then collated as the hospital document by the AMSC after modifying it with the hospital antibiogram. An implementation study to ascertain acceptance and use by the prescribers was performed before it was reviewed and updated as version 2.0 in 2024. Four clinical departments in the hospital contributed to version 1.0 while all clinical departments in the hospital contributed to version 2.0.
- Antibiograms were developed by the Microbiology Department to guide development of antibiotic policy and guidelines.
- The hospital's Microbiology Handbook was updated to serve as a tool for diagnostic stewardship.
- Manuals for induction and in-service AMS training were developed, based on the content of the WHO and NCDC training documents. The induction manual guides the training of newly employed healthcare professionals, while the in-service manual guides the training of AMSTs, antibiotic champions and the continuous training of regular staff.
- Manual of infection control policies and procedures was developed to guide infection prevention and control practices in the hospital. This was done in 2021.
- AMS action plan was also developed in 2021 and updated in 2022 and 2024. Development of this document and reviews were based on a SWOT analysis performed with the CwPAMS template annex 1 Annex 1 CwPAMS AMS Assessment Tool Oct 2022.docx

Advocacy visits to management:

The first advocacy visit paid to the management by the AMSC ensured the support of the hospital management throughout the project. The Chief Medical Director gave his full support and showed great interest in the stewardship programme of the hospital. He also emphasized that the ASP and all AMS interventions had to be discussed and understood by all hospital staff. The involvement of the management ensured a smooth running of the programme and their support during the project activities.

During this visit, seven requests were made, which include among others that management ensures the mandatory use of the just formulated antibiotic policy and guidelines by all prescribers of antibiotics in the hospital and turn the hospital policy into directives for the departments. Other requests are listed in Box 3.

Trainings:

Trainings were conducted both in-person and virtually to encourage participation of other healthcare professionals in the country. The first set of trainings took place in 2022 and involved training of all staff on the principles and practices of AMS. In attendance were 79 members of the AMSC and AMSTs, prescribers (150 resident doctors and house officers, and 105 consultants/medical officers), 36 pharmacists, 42 nursing clinical tutors and 271 trained nurses. The sharing and intense discussions of the developed relevant policies and documents enabled the members of the AMSC and AMSTs, prescribers, nurses and pharmacists better understand their specific roles as antimicrobial stewards. While the members of the AMSC and AMST had the terms of reference clearly explained to them, all healthcare professionals were also able to discuss and accept their AMS roles.

The trainings were repeated in 2024 and included 75 members of the AMSC and AMST, 52 pharmacists (including interns), 173 nurses, 122 resident doctors, and 85 house officers. The trainings were concluded with a two-day behaviour change workshop and the inauguration of 50 Antibiotic Champions spread across departments.

Box 3: Advocacy requests made by Lagos University Teaching Hospital Antimicrobial Stewardship Committee (AMSC)

- Ensure the mandatory use of the formulated antibiotic policy and guidelines by all prescribers of antibiotics in the hospital.
- Turn the LUTH Antibiotics Policy into directives for the various departments.
- Support the capacity building of LUTH healthcare workers including the AMS committee and AMS teams for antimicrobial stewardship.
- Support motivation and information of the stakeholders especially LUTH management for AMS.
- Give authority to our stewardship interventions, especially prospective audit and antibiotic consumptions calculations by the Pharmacy Department.
- Approve strategies recommended by the Pharmacy Department to encourage the patronage of LUTH Pharmacy by all patients with respect to the purchase of antibiotics.
- Allocate protected time for AMS for LUTH staff in various wards and departments.

During the second advocacy visit to the hospital management, the following requests were made:

- Sign the action plan. The importance of this had been mentioned to the CMD, especially as he is an additional partner in the project. This is also in line with the suggestion of the CMD that the action plan must be discussed, understood and accepted by the hospital community.
- Convert the policy into administrative directives. This was recognised as needful during the training of the AMSTs.
- Address the issue of the cephazolin purchased by the hospital for surgical antibiotic prophylaxis which was yet to be used.
- Reports from AMSTs should be through HODs to the AMSC, copying the CMAC, to further point it out to HODs that AMS is everybody’s duty in departments.
- Support the Microbiology Laboratory, Pharmacy and AMS unit to iron out issues based on the suggestions of the consultants, action plan and antibiotics policy.

Box 4: Duties of Lagos University Teaching Hospital Antimicrobial Stewardship Teams (AMSTs)

- Prospective audit using a checklist prepared from the antibiotic policy/guidelines
- A one-hour weekly meeting for team members to review the findings of audit, interventions and plan activities.
- A five- to ten-minutes talk or presentation on rational antibiotic prescribing at every Wednesday clinical meeting in clinical departments.
- A monthly report of audit findings and stewardship activities to the departments, antimicrobial stewardship committee and office of the CMAC.
- Reports of compliance rates to antibiotic guidelines and AMS interventions in the various clinical departments as a means of monitoring and evaluation.

Duties of Antimicrobial Stewardship Teams:

The AMSTs in 10 departments have been performing their roles and conducting prospective audit with intervention and feedback (PAIF) in both in-patients and out-patients (Box 4). Every week, they performed reminders at clinical meetings in their various departments (a minimum of 5-10 minutes) to reinforce AMS principles. To reduce the work burden on healthcare professionals, medical students who had already been trained during their clinical postings initiated prospective audits, while AMST members performed the intervention and feedback to the prescribers. This also afforded the students early training and practice in rational antibiotic use.

Reports of Antimicrobial Stewardship Teams:

The AMSTs in the different departments began submitting their periodic reports. Monthly reports of activities by the AMSTs were sent to the AMSC and ultimately to LUTH management and the Nigerian Centre for Disease Control (NCDC). These reports highlighted the progress the teams had made. As at March 2022, the new stewardship teams in the departments of Medicine, Surgery, Obstetrics and Gynaecology, Family Medicine and ICU had their inaugural meetings.

Medical students rotating in Medicine, Surgery and Obstetrics & Gynaecology were trained on prospective audit and feedback. Weekly 5-minute presentations on rational antibiotics use by members of the team was started in the Departments of Paediatrics, Medicine and Surgery. Following the conduct of the workshop on behaviour change, a WhatsApp group chat was created, with more than 30 nurses currently on the platform. This group consists of nurses in clinical and teaching units, LUTH nurses, and nursing students. A blog was also created for the Antibiotic Champions for new AMS information and participation.

AMS action plan:

The most recent AMS action plan (3.0) was developed in January 2025, for a 3-year period and is scheduled for update in 2028. It was written based on the CwPAMS template (Annex 3 CwPAMS AMS action plan template). The components include; (i) Objectives, (ii) Leadership commitment, accountability and responsibility, AMS actions, education and training, monitoring and surveillance, reporting feedback within the healthcare facility, (iii) Identified gaps, (iv) Agreed actions, (v) How will the actions be carried out? (vi) Who is/are responsible? and (vii) Agreed time frame to complete the actions.

The action plan was based on a situation analysis and was developed in discussion with the relevant stakeholders. The strengths weaknesses, opportunities and threats to the locally agreed actions were also considered. The action plan was finally presented to the AMSC and the hospital management team. The AMSC has the responsibility for overseeing the delivery of the agreed actions.

Implementation of antibiotic consumption (AMC) calculation by pharmacists:

The Pharmacy Department began calculating antibiotic consumption (AMC) as they appreciated the importance of this during the training sessions, and began collecting antibiotics consumption data in the in-patient pharmacy units.

A pharmacist who obtained the Africa Leadership Fellowship (ALF-A) was able to train other pharmacists, including 46 interns, on collecting AMC data (12). This facilitated improvement and consistency in the process. The antibiotics consumption calculation was then carried out periodically in all the in-patient units, to determine the Defined Daily Dose (DDD).

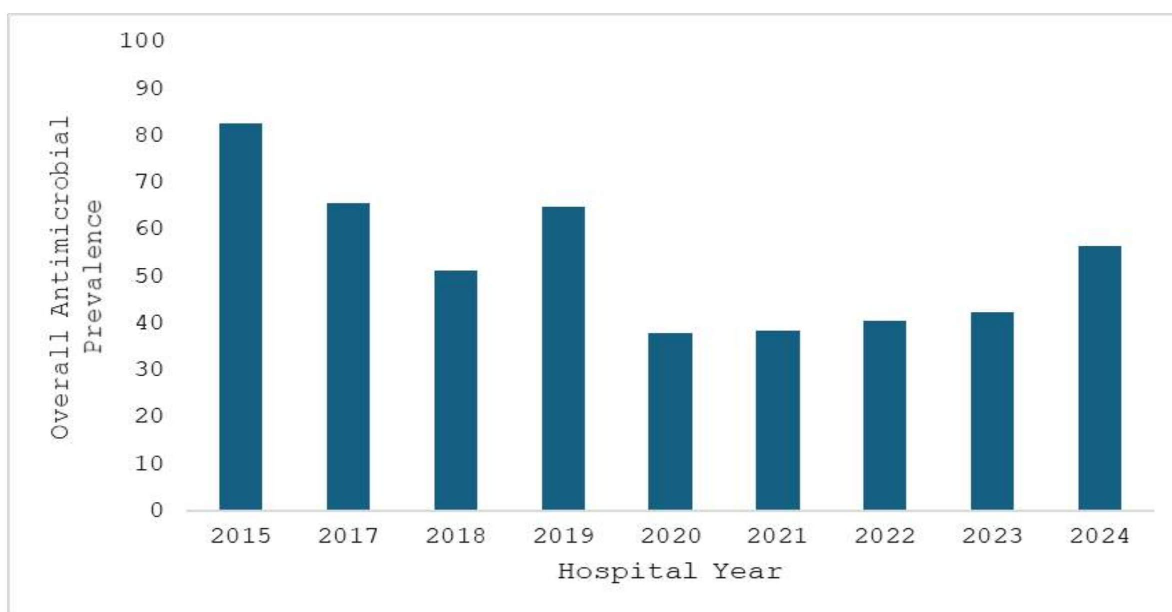

Fig 3: Annual Antimicrobial Use Prevalence rates in Lagos University Teaching Hospital, Nigeria (2015 – 2024)

Impact of Global-Point Prevalence Survey (G-PPS) and AMS actions in LUTH:

The highest antimicrobial use prevalence of 82.52% in LUTH was recorded in 2015 when there were no AMS actions introduced after conducting GPPS. By 2017, when awareness had been raised about AMS being a major strategy to mitigate the challenges of AMR, the antimicrobial prescribing rates reduced from 82.52% in 2015 to 65.50% in 2017. Following this, there has been a steady decline in AMU prevalence in subsequent years till 2020 after which the rates remained relatively steady till 2023 (Fig 3).

AMS actions started with one department in 2018 and had spread to four other departments by 2021. The actions included prospective audit with intervention and feedback (PAIF) and writing the hospital antibiotic policy. PAIF started in one department and gradually spread to five other clinical departments. Antibiotic consumption calculation is now performed in all in-patient wards.

Behaviour change workshops and Antibiotic Champions:

There were three workshops to address the issue of resistance to change. One was for people on prescribing pathways (prescribing, dispensing and or administering anti microbials). Workshop two was for members of AMSTs in all departments while workshop three was for appointed Antibiotic champions. The stakeholders for training included ward nursing leaders, members of the quality improvement committee and healthcare professionals (doctors, dentists, nurses, pharmacists) voted and appointed AMS Champions by their Heads of Departments.

Each of the workshops lasted 2.5 hrs and were moderated by behaviour change experts, who used the Capability, Opportunity and Motivation – Behavior (COM-B) model of behaviour change to explain the three components (capability, opportunity and motivation) to behaviour change (13). They covered motivating topics such as changing practice (mine and others), why the AMST is hard to action/what we can do, and having behaviour change conversations about AMS. The last workshop ended with a list of AMS actions populated by the champions themselves. The AMS Champions were inaugurated with badges and charged with the responsibility of motivating colleagues in their various departments to perform AMS.

Stakeholders engagement:

To sustain AMS activities, there was continuous advocacy to the hospital management to lead the way in institutionalising AMS policies. One key achievement was the approval of protected time for AMS activities, allowing healthcare staff to dedicate time to stewardship without compromising clinical duties. AMS initiatives were integrated into weekly departmental clinical meetings, which fostered continuous awareness and discussion of best practices among prescribers. Implementation was further strengthened by the 50 Antibiotic Champions appointed across departments, serving as focal points for AMS advocacy and intervention.

The AMS stakeholders meeting in 2022 had representatives of the Nigeria Centre for Disease Control (NCDC), Nigeria Institute of Medical Research (NIMR), Nigeria Medical Association (NMA), Pharmaceutical Society of Nigeria (PSN), and National Association of Nurses and Nurse Midwives (NANNM). Stakeholders were enlightened on the benefits of student involvement in AMS. The constraints

laboratory and pharmacy use to AMS were also identified, and possible solutions to improving these were discussed. Most of these stakeholders and organisations were also represented at the 2024 research dissemination meeting.

Discussion:

Antimicrobial stewardship had started in our hospital as far back as 2017, although there were challenges noticed mostly as resistance and unacceptability of perceived 'new' responsibilities by the healthcare professionals in the hospital. The CwPAMS 1.5 project enabled us to assess the gaps in our ASP. We were able to fill enough gaps to institutionalise AMS in our hospital, such that by 2022, colleagues who had earlier assumed AMS as a research project that would soon be completed, accepted it as an institutional improvement program.

Essentially, the CwPAMS 2.0 helped to ensure AMS activities and actions were performed to strengthen AMS activities in the hospital, and to build the capacity of the healthcare workers in AMS. This prepared the hospital for the Global Antimicrobial Stewardship Accreditation Schemes (GAMSAS) of the British Society for Chemotherapy (BSAC) at level 2, which further entrenched our ASP as the global certification was celebrated by all.

Lessons learnt:

Strategic training of staff enabled orientation of our healthcare professionals towards stewardship activities (14). Since the inception of our hospital ASP, we have used education as an AMS intervention. However, these had been done on an ad-hoc basis. To keep raising awareness of AMS, it must be done continuously and should be included in the routine programmes in the yearly plan for the hospital.

Another step in the right direction was the inclusion of presentations on AMS and rational antibiotic use in the weekly departmental ground rounds. Continuous training helps healthcare professionals better grasp their stewardship responsibilities, and this will continue to have a positive impact on their prescribing practices.

In 2019, we were able to demonstrate that medical students are capable of performing audits (13). With the hospital currently understaffed, medical students could play an essential role in AMS activities. They have been our auditors for prospective audits with intervention and feedback which is our hospital core AMS strategy. When trained, they also participate in the G-PPS as data collectors. These students have benefited from these activities by getting firsthand exposure to AMS activities in the hospital setting and antibiotics prescribing practices, in addition to the training they received. Hence, they have become equipped to tackle AMR even before they begin their practice. As future healthcare professionals, they already have the knowledge and good attitude towards AMS, therefore putting that into practice will be easier.

The support of management in hospital AMS activities proved to be a very crucial step in ensuring successful interventions and implementation of ASPs in line with what has also been documented (1). The relationship built with the hospital administration helped improve the acceptance of AMS activities throughout our facility.

Challenges:

Prospective audit stopped a few times due to industrial action in the hospital that affected the students who were the auditors. This would necessitate a planned backup for periods when students are absent from clinical postings and from wards where medical students do not rotate through during their professional training.

Calculating antibiotic consumption was challenging for the pharmacists before they were trained by the ALFA Fellow. Additionally, due to the absence of computers for data collection in various pharmacy units, the performance of this activity was slow. A hospital electronic medical records (EMR) if available would ensure this is done continually. However, our hospital EMR is still in development.

Some AMSTs have not been submitting reports regularly, making it difficult to determine the level of stewardship in their units. This is attributed to inadequate manpower, which is a complaint common to all departments and units of the hospital. Carrying out AMS activities as stewards is time and effort consuming, and if there is inadequate manpower, the available personnel could find it challenging, despite their best efforts. This to a large extent could be remedied by an operational EMR. Incorporating AMS data and interventions into hospital EMR has been reported to improve prescribing practices in hospitals as checks are put in place when prescribing antibiotics (15). The Lagos University Teaching Hospital and indeed, Nigeria as a country will benefit from adopting this as a national strategy.

Way forward:

The CwPAMS projects addressed some of the gaps we identified in our ASP and we performed many AMS interventions that have strengthened our programme. To continue in this direction, we have concerns that need to be resolved in the future. The most recent action plan must be diligently executed. To build on the success and progress achieved,

we need to continually measure the impact of the programme. We will continue to report this periodically using the Global-PPS, prospective audit with intervention and feedback, and antibiotic consumption calculations correlated with antibiotic resistance rates (16,17).

Raising awareness of AMS among present and future healthcare professionals must continue by training health workers at induction and in-service at regular stipulated intervals. All medical students should be included in AMS activities by incorporating AMS at all levels of their preclinical and clinical trainings. Currently, the basic principles of AMS are included in the undergraduate curriculum for medical students, and this would hopefully be replicated in the curricula of our pharmacy and nursing students.

Antimicrobial stewardship programme is well recognized as a behaviour change programme and the topmost AMS barrier in our hospital had been identified as 'resistance to change', hence our investment in the behaviour change workshops. Subsequent monitoring and evaluation of the champions will reveal their success or how we can further improve AMS practices (17).

Monthly correlations of AMC rates with AMR have to be performed and the results and implications should be communicated to the prescribers to help improve their prescribing practice. Hijazi et al., (14) in a review of the challenges and opportunities of AMS in resource-high and resource-limited countries, noted that while sustained engagements are required for changes in prescribing habits of health-care professionals, they advocated for population-level stewardship interventions and investment in structural factors to aid ASP implementation.

The CwPAMS 1.5 and 2.0 helped to improve the ASP of our hospital. It witnessed the support and approval of hospital administration and staff, as well as the involvement of aspiring medical professionals (students). We will keep expanding on these successes and be a positive example for other hospitals to follow and learn from.

Conclusion:

This is a descriptive report of the AMS efforts in our hospital. The CwPAMS 1.5 project enabled us to assess the gaps in our ASP and we were able to fill enough gaps to institutionalize AMS in our hospital by 2022. The CwPAMS 2.0 project prepared our hospital for the GAMSAS accreditation and further helped to entrench the programme.

These projects highlight how structured interventions, advocacy, and continuous training can successfully institutionalize AMS in a resource-limited setting. The collaboration between AMS teams, hospital management, and policymakers was instrumental in strengthening AMS policy enforcement, expanding capacity-building initiatives, and integrating stewardship practices into routine hospital operations. However, sustained commitment particularly in IT infrastructure is required to retain the gains. By continuing to evaluate, refine, and expand AMS interventions, LUTH can serve as a model for AMS implementation in Nigeria and other low-and-middle-income-countries (LMICs).

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Contributions of authors:

All authors were involved in the study concept and design. TAK, FIB, OCA and OOO produced the initial draft and CEB was involved in data collection and analysis. All the authors interpreted the findings, critically reviewed and approved the final manuscript.

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Conflict of interests:

No conflict of interests is declared.

Availability of data and materials

The datasets used and/or analysed during the current study are within the manuscript and are available from the corresponding author upon reasonable request.

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References:

1. Pulcini, C., Morel, C. M., Tacconelli, E., et al. Human resources estimates and funding for antibiotic stewardship teams are urgently needed. Clin Microbiol Infect. 2017; 23 (11): 785-787. doi: 10.1016/j.cmi.2017.07.013.
2. Cook, P. P., and Gooch, M. Long-term effects of an antimicrobial stewardship programme at a tertiary-care teaching hospital. Int J Antimicrob Agents. 2015; 45(3): 262–267. doi: 10.1016/j.ijantimicag.2014.11.006.
3. Wang, H., Wang, H., Yu, X., et al. Impact of antimicrobial stewardship managed by clinical pharmacists on antibiotic use and drug resistance in a Chinese hospital, 2010–2016: a retrospective observational study. BMJ Open. 2019; 9 (8): e026072. https://bmjopen.bmj.com/content/9/8/e026072
4. Kpokiri, E. E., Taylor, D. G., and Smith, F. J. Development of antimicrobial stewardship programmes in low and middle-income countries: A mixed-methods study in Nigerian hospitals. Antibiotics. 2020; 9 (4): 204 doi: 10.3390/antibiotics9040204
5. Oduyebo, O. O., Olayinka, A. T., Iregbu, K. C., et al. A Point Prevalence Survey of Antimicrobial Prescribing in Four Nigerian Tertiary Hospitals. Ann Trop Pathol. 2017; 8: 42-46 doi:10.4103/atp.atp_38_17
6. Oduyebo, O., Oshun, P., Gbotolorun, O., et al. LUTH Antimicrobial stewardship committee. Antimicrobial prescribing practices in a Nigerian Teaching Hospital: making a case for antimicrobial stewardship. Nig Qt J Hosp Med. 2017; 27 (1): 695-702
7. Abubakar, U. Antibiotic use among hospitalized patients in northern Nigeria: a multicenter point-prevalence survey. BMC Infect Dis. 2020; 20: 86. doi: 10.1186/s12879-020-4815-4.
8. Ogunleye, O. O., Oyawole, M. R., Odunuga, P. T., et al. A multicentre point prevalence study of antibiotics utilization in hospitalized patients in an urban secondary and tertiary healthcare facilities in Nigeria: findings and implications. Expert Rev Anti Infect Ther. 2022; 20: 297–306. doi: 10.1080/14787210.2021.1941870
9. Fadare, J. O., Ogunleye, O., Illiyasu, G., *et al*. Status of antimicrobial stewardship programmes in Nigerian tertiary healthcare facilities: findings and implications. J Glob Antimicrob Resist. 2019; 17: 132–136. https://doi.org/10.1016/j.jgar.2018.11.025
10. Iregbu, K. C., Nwajiobi-Princewill, P. I., Medugu, N., et al. Antimicrobial stewardship implementation in Nigerian hospitals: gaps and challenges. Afr J Clin Exper Microbiol. 2021; 22 (1): 60-66. doi: 10.4314/ajcem.v22i1.8
11. Oshun, P. O., Roberts, A. A., Osuagwu, C. S., *et al*. Roll out of a successful antimicrobial stewardship programme in Lagos University Teaching Hospital Nigeria using the Global-Point Prevalence Survey. Afr J Clin Exper Micro- biol. 2021; 22 (2): 260-272. doi: 10.4314/ajcem.v22i2.19
12. Savieli, C., Gülpinar G., Rutter V, *et al*, Africa Leadership Fellowship Antimicrobial Stewardship (ALF-A) – insights on mentorship experiences. Int J Pharm Pract. 2024; 32 (suppl 2): ii48–ii49, https://doi.org/10.1093/ijpp/riae058.057
13. Hart J., Lucie Byrne-Davis L., Maltinsky W., et al. Developing CPD to Change Behaviour: In Training to change practice: Behavioural science to develop effective health professional education 2023; John Wiley and Sons, Ltd. onlinelibrary.wiley.com/ https://doi.org/10.1002/9781119833505.ch4.
14. Hijazi, K., Joshi, C., and Gould, I. M. Challenges and opportunities for antimicrobial stewardship in resource-rich and resource-limited countries. Expert Rev Anti Infect Ther. 2019; 17 (8): 621-634. doi:10.1080/14787210.2019.1640602.
15. Roberts, A. A., Fajolu, I., Oshun, P., et al. Feasibility study of prospective audit, intervention and feedback as an antimicrobial stewardship strategy at the Lagos University Teaching Hospital. Niger Postgrad Med J. 2020; 27: 54–58. doi: 10.4103/npmj.npmj_115_19.
16. Weihs, S. Antimicrobial Stewardship by Leveraging Electronic Medical Records. Mo Med. 2020; 117 (4): 338-340.
17. Alghamdi, S., Berrou, I., Bajnaid, E., et al. Antimicrobial Stewardship Program Implementation in a Saudi Medical City: An Exploratory Case Study. Anti- biotics (Basel). 2021; 10 (3): 280. doi:10.3390/antibiotics10030280.
18. Patel, J., Ransom, S., Bennet, C., Culloty, S., and Micallef, C. P03 An evaluation of Antibiotic Champions events in GP practices in Cambridgeshire and Peterborough Integrated Care System. JAC - Antimicrobial Resistance. 2024; 6 (Suppl 2): dlae136.007. https://doi.org/10.1093/jacamr/dlae136.007

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Original Article **Open Access**

Bacterial profile and antibiogram of clinical isolates in a tertiary healthcare facility in northeast Nigeria: Initial steps towards developing local antibiotic guidelines and antimicrobial stewardship programme

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Abstract:

Background: Antibiograms and antibiotic guidelines are important tools for appropriate prescribing practices in combating the antimicrobial resistance (AMR) challenge. They serve as a prelude to an evidence-based Antimicrobial Stewardship (AMS) program, which is necessary for better Infection Prevention and Control (IPC) activities, especially in low-resource settings like Nigeria. This study determined the bacterial profile and antibiogram of clinical isolates in a tertiary healthcare facility in Gombe, northeastern Nigeria.
Methodology: This was a 4-year retrospective descriptive analysis of bacterial isolates from in and outpatient clinical specimens submitted to the Medical Microbiology Laboratory of the hospital between January 2019 to December 2022. Specimens were cultured for bacterial isolation and phenotypic identification using conventional techniques. Antibiotic susceptibility test was performed on each isolate by the Kirby Bauer disc diffusion method.
Results: A total of 15,457 bacteria were isolated over the 4-year period and include *Staphylococcus aureus* (6604, 42.72%), *Klebsiella* species (2382, 15.41%), *Escherichia coli* (2140, 13.84%), *Pseudomonas* species (1429, 9.25%), *Proteus* species (469, 3.03%) and *Enterococcus* species (215, 1.39%). The overall susceptibility (antibiogram) of all the bacterial isolates to commonly used antibiotics over the 4-year period was 59.0% for gentamicin, 54.5% for levofloxacin, 50.6% to ciprofloxacin, 48.5% to ceftriaxone, 48.5% to ceftazidime, and 41.9% to amoxicillin/clavulanate. Resistance rate was more than 50.0% for many of the tested antibiotics (ceftriaxone, ceftazidime, amoxicillin/clavulanate, and cefoxitin).
Conclusion: There was high level of resistance to many routinely used antibiotics tested in our facility. There is need for evidence-based AMS programmes hinged on local antibiotic guidelines for better patient safety and improved healthcare quality particularly in resource poor settings.

Keywords: Antibiogram; Antimicrobial Stewardship; Antibiotic Guidelines; Infection Prevention & Control

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Profil bactérien et antibiogramme d'isolats cliniques dans un établissement de soins de santé tertiaire dans le nord-est du Nigéria: premières étapes vers l'élaboration de directives locales sur les antibiotiques et d'un programme de gestion des antimicrobiens

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Résumé:

Contexte: Les antibiogrammes et les directives sur les antibiotiques sont des outils importants pour des pratiques de prescription appropriées dans la lutte contre le défi de la résistance aux antimicrobiens (RAM). Ils servent de prélude à un programme de gestion des antimicrobiens (GAM) fondé sur des données probantes, qui est nécessaire pour de meilleures activités de prévention et de contrôle des infections (PCI), en particulier dans les environnements à faibles ressources comme le Nigéria. Cette étude a déterminé le profil bactérien et l'antibiogramme des isolats cliniques dans un établissement de soins de santé tertiaire à Gombe, dans le nord-est du Nigéria.
Méthodologie: Il s'agissait d'une analyse descriptive rétrospective sur 4 ans des isolats bactériens provenant d'échantillons cliniques hospitaliers et ambulatoires soumis au laboratoire de microbiologie médicale de l'hôpital entre janvier 2019 et décembre 2022. Les échantillons ont été cultivés pour l'isolement bactérien et l'identification phénotypique à l'aide de techniques conventionnelles. Un test de sensibilité aux antibiotiques a été effectué sur chaque isolat par la méthode de diffusion sur disque de Kirby Bauer.
Résultats: Au total, 15 457 bactéries ont été isolées sur la période de 4 ans et comprennent *Staphylococcus aureus* (6604, 42,72%), des espèces de *Klebsiella* (2382, 15,41%), *Escherichia coli* (2140, 13,84%), des espèces de *Pseudomonas* (1429, 9,25%), des espèces de *Proteus* (469, 3,03%) et des espèces d'*Enterococcus* (215, 1,39%). La sensibilité globale (antibiogramme) de tous les isolats bactériens aux antibiotiques couramment utilisés sur la période de 4 ans était de 59,0% pour la gentamicine, 54,5% pour la lévofloxacine, 50,6% pour la ciprofloxacine, 48,5% pour la ceftriaxone, 48,5% pour la ceftazidime et 41,9% pour l'amoxicilline/clavulanate. Le taux de résistance était supérieur à 50,0 % pour de nombreux antibiotiques testés (ceftriaxone, ceftazidime, amoxicilline/clavulanate et céfoxitine).
Conclusion: Il y avait un niveau élevé de résistance à de nombreux antibiotiques couramment utilisés testés dans notre établissement. Il est nécessaire de mettre en place des programmes de gestion des antibiotiques fondés sur des données probantes et s'appuyant sur des directives locales en matière d'antibiotiques pour améliorer la sécurité des patients et la qualité des soins de santé, en particulier dans les milieux pauvres en ressources.

Mots-clés: Antibiogramme; Gestion des antimicrobiens; Directives sur les antibiotiques; Prévention et contrôle des infections

Introduction:

Overuse of empirical broad-spectrum antibiotics and inadequate antimicrobial stewardship (AMS) programs at all levels have significantly contributed to the emergence and spread of multidrug-resistant organisms (MDROs) (1). The global impact of MDROs has detrimentally affected worldwide infection prevention and control (IPC) efforts, patient safety, and healthcare quality with severe repercussions particularly in low-resource settings, manifesting in significant healthcare challenges. Availability of bacterial profiles and their antibiograms from clinical isolates is necessary for the control of resistance to antimicrobial agents especially in developing countries (2,3).

Apart from being a component of the AMS programme, the antibiogram provides important information about the sensitivity/ resistance pattern in particular settings, enabling an evidence-based empirical use of antibiotics. This is especially important in many low-and-middle-income-countries (LMICs) where patients are unable to afford payment for routine microbiological investigations or the facilities for such are not available (4,5). Results of studies on antibiotic stewardship programmes in Africa revealed lack of institutional annual antibiograms as one deficiency in many countries (6,7).

Antimicrobial Resistance (AMR) commonly results from their misuse and abuse across human, agricultural/environmental and veterinary healthcare in addition to poor AMS programmes/strategies including inadequate surveillance and policies/guidelines (8-10). Resistance to clinically important Gram-positive bacteria such as *Staphylococcus aureus* including methicillin resistant *S. aureus* (MRSA), *Streptococcus pneumoniae*, *Enterococcus* spp, *Corynebacterium* spp and *Streptococcus agalactiae*, and Gram-negative bacteria such as *Escherichia coli, Klebsiella* spp, *Pseudomonas, Acinetobacter* spp, *Proteus, Morganella, Citrobacter* spp, *Enterobacter* spp, *Salmonella* spp, and *Shigella* spp to commonly available antibiotics have been widely reported in sub-Saharan African countries (11-13). This constitutes a major public health challenge with heavy financial burden on both patients and healthcare providers (14,15).

In some developed countries, deployment of effective IPC measures has led to reduction in the prevalence of key MDROs such as MRSA in contrast to sub-Saharan Africa where the burden of infectious diseases

is still unacceptably high (16,17). In Nigeria, a 2.3-fold increase in MRSA prevalence was reported in association with several infections including osteomyelitis, bloodstream, skin/soft tissue/wound, surgical sites, respiratory, and urinary tract infections over a five-year period (18). Antibiograms play a key role in improving the appropriateness of empirical antibiotic therapy and reducing the emergence and spread of AMR in any given society or institution (19). This study presents a four-year review of bacterial profiles and antibiogram of clinical isolates in Federal Teaching Hospital Gombe, as a prelude to development of local antibiotic policy for evidence-based AMS programme in the hospital.

Materials and method:

Study area:

This study was conducted in a 555-bed Federal Teaching Hospital Gombe (FTHG), a tertiary health facility in northeastern Nigeria. Gombe State is located at the centre of northeastern Nigeria and FTHG receives and manages patients from all neighbouring states and beyond. The hospital enjoys the complement of virtually all common medical/surgical specialties.

Ethical consideration:

This study used existing laboratory records (secondary data), hence, there was no risk of physical harm to the patients. The data were de-identified for confidentiality and privacy of patients. Ethical approval was obtained from the Research and Ethics Committee of FTHG, before the commencement of the study.

Clinical isolates analysed from routine specimens:

Routine clinical specimens (urine, sputum, blood, aspirates, swabs, and biopsies) collected from all in and outpatients of all age groups with suspected clinical infections and processed in the Medical Microbiology laboratory of FTHG from January 2019 to December 2022, yielded the isolates analysed in this study.

Culture isolation and identification:

The specimens were routinely collected according to standard procedures in appropriate specimen containers, timely transported to the laboratory and processed immediately by conventional culture isolation methods. All urine specimens were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) and Blood Agar (BA) plates, while sputum, blood, seminal fluid and swabs/aspirates/biopsies were all inoculated on MacConkey, Blood and Chocolate agar plates. All plates were incubated aerobically at 37°C for 16-24 hours. Isolates were identified by colonial morphology, Gram-reaction and conventional biochemical test schemes.

Antibiotic susceptibility testing:

Antibiotic susceptibility testing (AST) of the isolates was done using modified Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar plates and results were interpreted according to the 2019 Clinical and Laboratory Standard Institutes (CLS1) guidelines (16). The antibiotic discs (Oxoid UK) include amoxicillin/clavulanate (20/10 µg), ceftriaxone (30µg), ceftazidime (30µg), cefoxitin (30µg), gentamicin (10µg), levofloxacin (5µg), ciprofloxacin (5µg), and erythromycin (15µg).

Results:

Bacterial profiles:

A total of 15,457 bacteria were isolated over the 4-year period of the study; 2019 (n=4,025), 2020 (n=3,978), 2021 (n=3,885) and 2022 (n=3,569). Majority were from urine (9,504, 61.49%), aspirates/swabs/biopsies (3,727, 24.11%), blood (1238, 8.01%) and sputum (988, 6.39%). The commonly isolated bacteria are *S. aureus* (6,604, 42.72%), *Klebsiella* spp (2,382, 15.41%), *Escherichia coli* (2,140, 13.84%), *Pseudomonas* spp (1,429, 9.25%), *Proteus* spp (469, 3.03%) and *Enterococcus* spp (215; 1.39%). Other Gram-negative and Gram-positive bacteria constituted 1,330 (8.60%), and 897 (5.80%) respectively. The distributions of the isolates by year of identification and specimens of origin is highlighted in Table 1.

Antibiogram:

The overall susceptibility to common antibiotics tested on all the bacteria could be summarised as gentamicin (59.0%), levofloxacin (54.5%), ciprofloxacin (50.6%), ceftriaxone (48.5%), ceftazidime (48.5%), and amoxicillin/clavulanate (41.9%). The susceptibility of all *S. aureus* isolates to cefoxitin was 44.5%, indicating 55.5% MRSA rate. Of all the Gram-positive bacteria tested, only 36.9% were susceptible to erythromycin.

Table 1: Distributions of isolates by year of identification and specimens of origin

Specimen	**Organism**	**2019**	**2020**	**2021**	**2022**	**Total (%)**
Urine	*Staphylococcus aureus*	888	1013	1214	973	4088 (43.01)
	Escherichia coli	388	519	288	425	1620 (17.04)
	Klebsiella species	347	352	534	356	1589 (16.71)
	Other Gram negatives	273	171	216	165	825 (8.68)
	Pseudomonas species	206	148	259	183	796 (8.38)
	Other Gram positives	69	64	48	56	237 (2.49)
	Enterococcus species	55	39	47	24	165 (1.74)
	Proteus species	54	63	19	48	184 (1.94)
		2280	2369	2625	2230	9504 (100.0)
Swabs/ Aspirates/ Biopsies	*Staphylococcus aureus*	457	543	357	343	1700 (45.61)
	Klebsiella species	132	97	55	095	379 (10.17)
	Pseudomonas species	127	108	117	126	478 (12.82)
	Other Gram positives	107	90	41	55	338 (9.07)
	Proteus species	82	89	18	70	259 (6.95)
	Escherichia coli	76	128	76	75	355 (9.53)
	Other Gram negatives	76	37	35	71	219 (5.88)
	Enterococcus species	20	7	1	16	44 (1.18)
		1077	1099	700	851	3727 (100)
Blood	*Staphylococcus aureus*	269	136	197	171	773 (62.44)
	Other Gram positives	31	10	24	26	91 (7.35)
	Klebsiella species	11	15	17	10	53 (4.28)
	Proteus species	6	6	1	0	13 (1.05)
	Escherichia coli	4	4	19	23	50 (4.04)
	Other Gram negatives	2	31	65	7	175 (14.14)
	Enterococcus species	0	1	2	2	5 (0.40)
		337	227	365	309	1238 (100.0)
Sputum	*Klebsiella* species	132	133	042	54	361 (36.54)
	Other gram positives	76	79	71	50	276 (27.93)
	Pseudomonas species	42	15	20	0	77 (7.79)
	Other Gram negatives	39	14	17	41	111 (11.23)
	Escherichia coli	26	26	30	33	115 (11.64)
	Staphylococcus aureus	15	12	15	1	43 (4.35)
	Enterococcus species	1	0	0	0	1 (0.10)
	Proteus species	0	4	0	0	4 (0.40)
		331	283	195	179	988 (100.0)

The distribution of isolates and their percentage susceptibility to common antibiotics and year of isolation is highlighted in Table 2 and Fig 1. The trend in susceptibility to the common antibiotics for *E. coli*, showed that about 70% were susceptible to gentamicin in 2019. But in the subsequent years (2020, 2021 and 2022), the susceptibility dropped to 51%, 47.7% and 50.5% respectively. Susceptibility of *E. coli* to ceftriaxone was 64.2% in 2019, 35.4% in 2020, 43.6% in 2021 and 33.3% in 2022. However, the susceptibility of the bacterium to ciprofloxacin over the years was 49.7% (2019), 34.4% (2020), 28.1% (2021) and 42.8 (2022).

The susceptibility *of Pseudomonas* spp to commonly used antibiotics were; 53.9% (2019), 83.3% (2020), 22.2% (2021), and 38.9% (2022) for ceftazidime; and 63.5% (2019), 50.3% (2020), 50.0% (2021), and 48.4% (2022) for gentamicin, while for ciprofloxacin, the susceptibility was 70.3% (2019) 52.7% (2020), 49.7% (2021) and 45.4% (2022).

Staphylococcus aureus isolates in the study was steadily susceptible to gentamicin for the 4-year period, while for ciprofloxacin, susceptibility of 38.9%, 24.6%, 40.1% and 52.8% were observed in 2019, 2020, 2021 and 2022 respectively. The trends in susceptibility to other antibiotics were as shown in Figs 2, 3 and 4.

Table 2: Antibiogram (Percentage susceptibility) of the bacterial isolates to common antibiotics based on years

Year	Bacteria	Number	Percentage (%)							
			CN	AMC	FOX	CRO	CIP	LEV	CAZ	E
2019	*Staphylococcus aureus*	1629	66.5	NA	51.0	NA	38.1	33.2	NA	32
	Klebsiella pneumoniae	622	62.0	45.5	NT	55.4	62.5	29.4	NT	NA
	Escherichia coli	494	69.5	45.1	NT	64.2	49.7	25.4	NT	NA
	Proteus spp	142	63.2	48.9	NT	76.4	68.3	100	NT	NA
	Pseudomonas aeruginosa	389	63.5	NA	NA	NA	70.3	57.3	53.9	NA
	Enterococcus spp	076	NA	AN	NT	NA	68.8	45.7	NA	50.0
	Other Gram negatives	390	78.8	48.5	NT	76.6	71.2	54.2	NT	NA
	Other Gram positives	283	73.0	NT	NT	NT	65.4	45.7	NT	46.2
2020	*Staphylococcus aureus*	1704	59.5	NA	42.0	NA	24.6	28.4	NA	21.7
	Klebsiella pneumoniae	597	53.8	36.8	NT	38.8	53.5	37.0	NT	NA
	Escherichia coli	677	51.2	22.1	NT	35.4	34.4	14.5	NT	NA
	Proteus spp	162	66.1	59.1	NT	63.6	53.1	50.5	NT	NA
	Pseudomonas spp	295	50.3	NA	NA	NA	52.7	45.1	83.3	NA
	Enterococcus spp	047	NA	NA	NA	NA	63.2	70.4	NA	33.3
	Other Gram negatives	253	73.8	57.3	NT	66.0	71.7	66.8	NT	NA
	Other Gram positives	243	68.2	78.5	NT	NT	68.6	72.5	NT	53.2
2021	*Staphylococcus aureus*	1783	57.8	NA	37.3	NA	40.1	36.6	NA	26.6
	Klebsiella spp	648	46.6	43.7	NT	23.1	45.4	41.6	NT	NA
	Pseudomonas spp	436	50.0	NA	NA	NA	49.7	53.0	22.2	NA
	Escherichia coli	413	47.7	33.8	NT	43.6	28.1	57.1	NT	NA
	Enterococcus spp	050	NA	NA	NA	NA	44.0	45.4	NA	30.7
	Proteus spp	038	63.6	25.0	NT	38.7	40.0	55.5	NT	NA
	Other Gram-negatives	333	54.8	32.8	NT	39.2	48.6	56.4	NT	NA
	Other Gram-positives	184	60.0	50.0	NT	NT	43.3	51.9	NT	22.2
2022	*Staphylococcus aureus*	1488	61.5	NA	47.6	NA	46.8	52.8	NA	34.5
	Escherichia coli	556	50.5	25.0	NT	33.3	42.8	46.1	NT	NA
	Klebsiella spp	515	52.2	27.6	NT	36.1	41.5	47.8	NT	NA
	Pseudomonas spp	309	48.4	NA	NA	NA	35.9	45.4	38.9	NA
	Proteus spp	118	40.9	39.9	NT	45.0	34.2	45.6	NT	NA
	Enterococcus spp	042	NT	NA	NA	NA	56.7	76.2	NA	49.9
	Other Gram-negatives	354	55.3	37.8	NT	40.1	49.2	60.8	NT	NA
	Other Gram-positives	187	51.9	NT	NT	NT	56.3	61.0	NT	47.5
		15457	**58.6**	**41.9**	**44.5**	**48.5**	**50.6**	**54.5**	**49.6**	**36.9**

NA (Not Applicable), NT (Not Tested), AMC (Amoxicillin/Clavulanate), CRO (Ceftriaxone), CAZ (Ceftazidime), FOX (cefoxitin), CN (Gentamycin), LEV (Levofloxacin), CIP (Ciprofloxacin), and E (Erythromycin); Other Gram-negatives: *Citrobacter* species, *Enterobacter* species, *Serratia* species, *Salmonella* species, *Acinetobacter* species, *Providentia, Morganella, Yersinia, Achromobacter, Moraxella and Stenotrophomonas* species. Other Gram-positives: *Streptococcus* species, *Bacillus* species and Diphtheroids

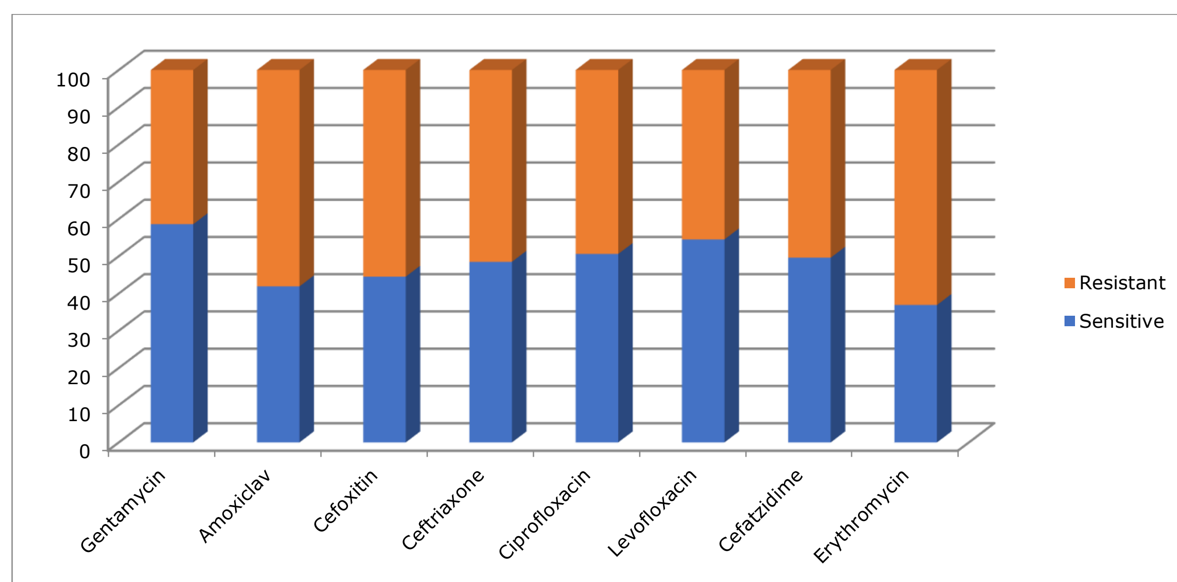

Fig 1: Overall percentage susceptibility (antibiogram) of the bacterial isolates to common first line antibiotics in the 4-year study period

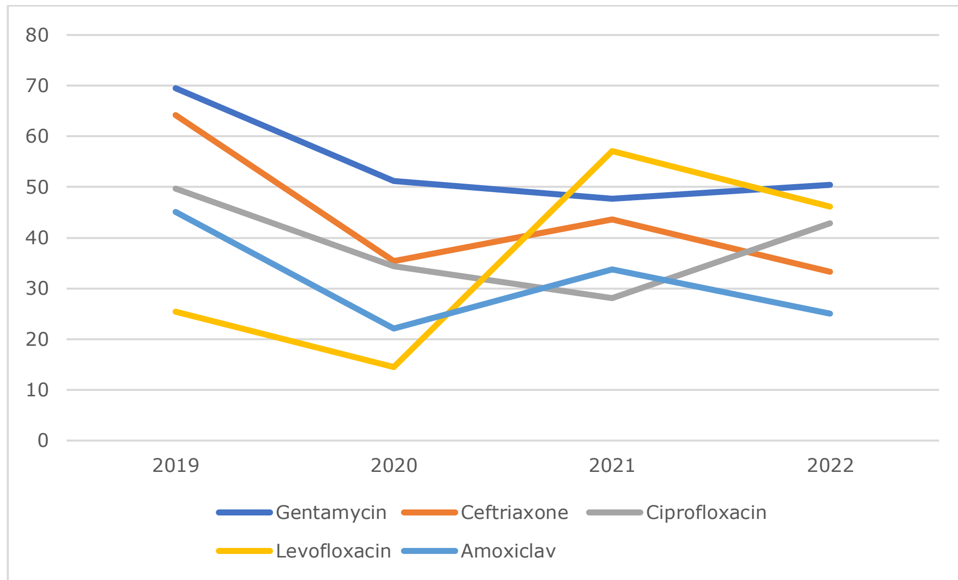

Fig 2: Trend of *Escherichia coli* isolates susceptibility to common antibiotics

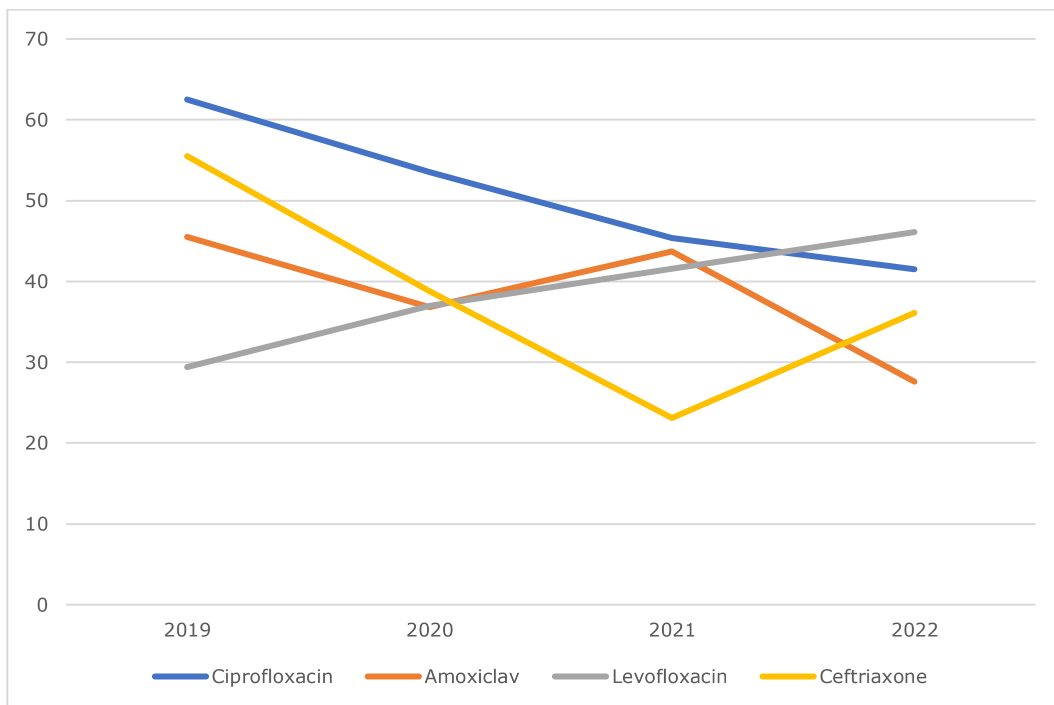

Fig 3: Trend of *Klebsiella* spp isolates susceptibility to common antibiotics

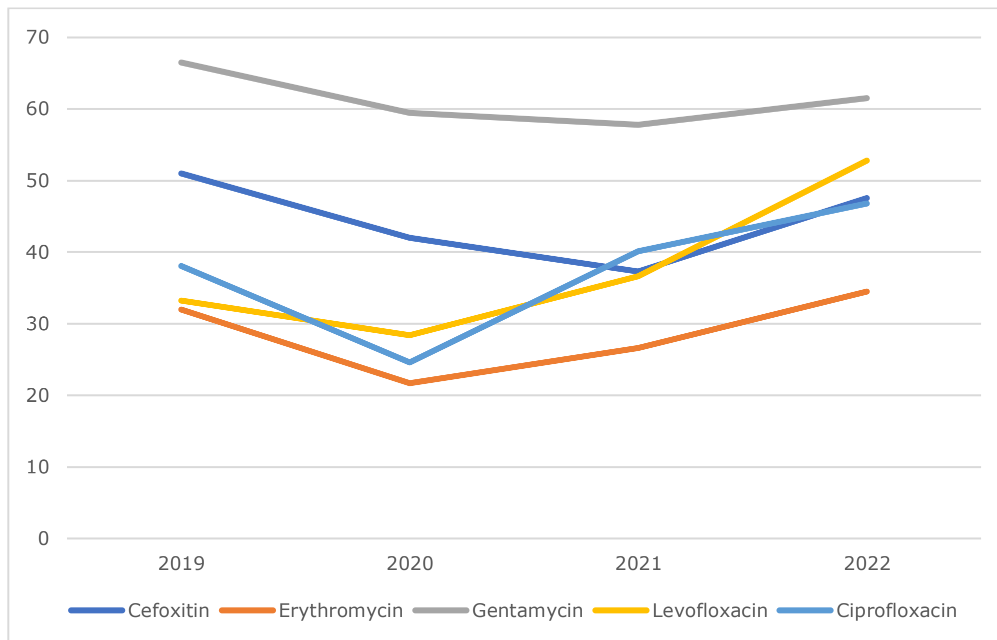

Fig 4: Trend of *Staphylococcus aureus* isolates susceptibility to common antibiotics

Discussion:

This study identified the most common bacterial isolates from clinical samples collected from patients with suspected clinical infections over a four-year period in our facility and their percentage susceptibility (antibiogram) to commonly used antibiotics. Urine (61.49%) and aspirates/swabs/biopsies (24.11%) contributed the highest number of bacterial isolates highlighting a similarity with the findings of an earlier report from the same centre which identified urine and aspirates/swabs/ biopsies as the specimens with highest yield of bacteria (17). Similar study carried out in Iraq, also showed that a significant proportion of the isolates (67.1%) and (19.6%) were respectively reported from urine and swabs/aspirates (18). Findings from a recent study conducted in Ghana were in keeping with this trend (19). Urinary tract infections are among the most common infectious diseases which reflect the isolates from urine being most predominant in most bacteriology laboratories (20).

The most isolated bacterium in this study was *S. aureus* (42.72%), which reflects the fact that staphylococci generally are associated with variety of infections and are equally common cause of infections in low-and-middle-income-countries (21). Among the first line antibiotics tested, *S. aureus* was most susceptible to gentamicin (61.3%) and least to erythromycin (28.7%). The susceptibility of *S. aureus* to erythromycin from a recent meta-analysis of studies conducted in Nigeria was about 53% (22). High prevalence of resistance to erythromycin in our study may be explained by the constitutive nature of the resistance expressed by staphylococci to erythromycin as previously reported in Gombe (23). Susceptibility to these antibiotics was low compared to that of a study in Asia that reported high susceptibility to gentamicin (86.2%) and erythromycin (83.3%) by staphylococci (24). A similar study in Algeria reported significantly high susceptibility to gentamicin of 92.3% by staphylococci isolated from clinical specimens (25). These variations in findings might not be unconnected with the openly observed rampant misuse and abuse of such antibiotics in our settings.

In this study, only 44.5% of the *S. aureus* was susceptible to cefoxitin, indicating that more than half of the *S. aureus* isolates were MRSA. Similar studies from different parts of Nigeria have reported varying figures on phenotypic MRSA prevalence of 65.4% and 22.6% (26,27). These variations may be due to genetic, environmental, methodology, and other factors. The prevalence of MRSA was 80% and 56.2% in studies conducted in Cameroon and Afghanistan respectively (28,29). The observed trend of susceptibility among isolated staphylococci showed that there was a steady increase in susceptibility to cefoxitin, gentamicin and ciprofloxacin in 2022.

Enterococcus spp, another Gram-positive bacteria, were observed to be most commonly susceptible to levofloxacin (59.4%), which is at variance with the results of a South Korean study where *Enterococcus* spp were highly sensitive (90.4%) to levofloxacin (30). This may be associated with the high carriage of multidrug resistant *Enterococcus* spp among individuals in Nigeria with levofloxacin

resistance being up to 34% in a study in south-western part of the country (31).

Klebsiella species were the second most prevalent bacteria isolated and the most common among the Enterobacterales in this study, but with overall low susceptibility to the commonly tested antibiotics. *Klebsiella* spp was identified as the second most common Gram-negative bacterial pathogen in a similar study conducted in Nigeria (32) while it was ranked first in another study from Ghana (33). The highest susceptibility of 53.7% was recorded for gentamicin and the least of 38.4% to amoxicillin/clavulanate by the *Klebsiella* isolates. These results are in keeping with reports from a study conducted in northwest Nigeria with susceptibility rates of 64.5% and 22.3% for *Klebsiella* spp to gentamicin and amoxicillin/clavulanate respectively (32). On the contrary, a study conducted in Taiwan reported susceptibility of *Klebsiella* spp to gentamicin and amoxicillin/clavulanate to be 84.8% and 89.1% respectively (34). In our study, *Klebsiella* spp demonstrated a continuous decline in susceptibility to ciprofloxacin from 2019 to 2022, while steep decrease to ceftriaxone from 2019 to 2021, stabilized and began to increase in susceptibility in 2022, with intermittent decline and increase in susceptibility to amoxicillin/clavulanate over the years under review.

Escherichia coli was the second most prevalent member of the order Enterobacterales in this study. This is not surprising as this pathogen is a known common causative agent of urinary tract infections and urinary isolates were the majority from the samples processed in the study. The highest susceptibility rate of 54.7% was recorded for gentamicin while a low rate of 31.5% was to amoxicillin/clavulanate. The low susceptibility of *E. coli* to amoxicillin/clavulanate may be due to widespread and non-rational use (availability to purchase without prescription) of this antibiotic commonly observed in our communities. A study assessing susceptibility of uropathogens in Africa reported a rate of 48% and 45% for *E. coli* to gentamicin and amoxicillin/clavulanate respectively (35). The susceptibility of *E. coli* to amoxicillin/clavulanate was 25.5% in a study conducted in Saudi Arabia (36). The overall trend of susceptibility of *E. coli* to the commonly used antibiotics showed a considerably high susceptibility to gentamicin, ceftriaxone, and ciprofloxacin in 2019, followed by steep decline in 2020/2021 and stabilizing in 2022. This may be linked to the overuse of these antibiotics for prophylaxis and empiric therapy, leading with the associated widespread resistance in the community and the hospital.

Pseudomonas spp showed similar susceptibility rates to all tested antibiotics; 53.1% to gentamicin, 52.2% to ciprofloxacin, 50.2% to levofloxacin and 49.6% to ceftazidime. The susceptibility rate to gentamicin has similarity to that reported in a study conducted in Saudi Arabia of 56.7% (37). However, susceptibility rates to ceftazidime (83.3%), levofloxacin (70.0%) and ciprofloxacin (76.7%) in the Saudi Arabia study were comparatively higher than the rates reported in our study. The overall trend of susceptibility to gentamicin continued to reduce steadily while the susceptibility to ceftazidime increased in 2020, this was immediately followed by a drastic decrease in 2021 and 2022. There was also associated decrease and increase in susceptibility to ciprofloxacin creating a zigzag pattern every year. This may not be unconnected to the difference in potency of the antibiotics used in these patients. The low susceptibility of *Pseudomonas* spp to these antibiotics may be due to their frequent use for empiric therapy and for prophylactic purposes in our facility. However, in a study conducted at Antwerp University Hospital (UZA), 91.4% sensitivity of *Pseudomonas* spp was reported to piperacillin/tazobactam (which we did not test in our study) and ceftazidime (38).

We had earlier reported a high level of antibiotic prescribing and over-reliance on empirical antibiotic therapy among healthcare practitioners in our centre and this region of the country (39,40). These were considered major drivers of AMR and threats to patient safety which require urgent attention in form of AMS programmes based on local evidence and policies/guidelines. Our study established a baseline profile of bacterial isolates and their antibiogram over a four-year period to serve as a prelude for an evidence-based hospital antibiotic policy. This will strengthen the AMS programme in the hospital and pave way for better IPC practices and improved patient safety and healthcare quality.

Our study is not without some limitations. It is a single centre study and cannot be generalized for the entire country. Additionally, manual biochemical methods were used for the identification of the bacteria. As such, it was impossible to completely identify and discountenance all duplicate isolates in the study as the data were retrospectively obtained from routine clinical isolates.

Conclusion:

Bacterial profiles and antibiogram of clinical isolates are important pre-requisites for evidence-based local antibiotic guidelines which are necessary for better antimicrobial stewardship programmes especially in low-resource settings. Findings from this study revealed a relatively high level and increasing trend of resistance to commonly used antibiotics by most of the tested bacteria. There is need to develop functional country-wide and

hospital-based antibiotic guidelines that can streamline empiric antibiotic therapy to reduce antimicrobial resistance and improve patient safety and healthcare quality.

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Contributions of authors:

MMM, MI, and GBG were involved in the study conception and design; MM, MWA, IEW, MIG, and MSC were involved in data collection and analysis; MM, JOF, MSC, HUF, and IEW were involved in interpretation of results; MM, JOF, and MI were involved in drafting of manuscript; and IEW, MWA, MI, and MSC were involved in revision of intellectual content. All authors reviewed and approved the final version of the manuscript submitted for publication.

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Conflict of interest

No conflict of interest is declared.

Availability of data and materials:

All datasets on which the conclusions of the manuscript rely; to be made available from the corresponding author on reasonable request from the editors.

References:

1. Malik, B., and Bhattacharyya, S. Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. Sci Rep. 2019; 9: 9788. doi: 10.1038/s41598-019-46078-y.
2. Laxminarayan, R., Duse, A., Wattal, C., et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013; 13: 1057–1098. doi: 10.1016/S1473-3099(13)70318-9
3. Alvarez-Uria, G., Gandra, S., and Laxminarayan, R. Poverty and prevalence of antimicrobial resistance in invasive isolates. Int J Infect Dis. 2016; 52: 59–61. doi: 10.1016/j.ijid.2016.09.026
4. Sono, T. M., Yeika, E., Cook, A., et al. Current rates of purchasing of antibiotics without a prescription across sub-Saharan Africa; rationale and potential programmes to reduce inappropriate dispensing and resistance. Expert Rev Anti Infect Ther. 2023; 21: 1025–1055.doi: 10.1080/14787210.2023.2259106.
5. Godman, B., Egwuenu, A., Wesangula, E., et al. Tackling antimicrobial resistance across sub-Saharan Africa: current challenges and implications for the future. Expert Opin Drug Saf. 2022; 21: 1089–1111. doi: 10.1080/14740338.2022.2106368.
6. Gebeyehu, E., Bantie, L., and Azage, M. Inappropriate Use of Antibiotics and Its Associated Factors among Urban and Rural Communities of Bahir Dar City Administration, Northwest Ethiopia. PLoS One. 2015; 10: e0138179. doi: 10.1371/journal.pone.0138179
7. Röing, M., Björkman, I., Eriksen, J., et al. The challenges of implementing national policies to contain antibiotic resistance in Swedish healthcare—A qualitative study of perceptions among healthcare professionals. PLoS One. 2020; 15: e0233236. doi: 10.1371/journal.pone.0233236
8. Grigoryan, L., Haaijer-Ruskamp, F. M., Burgerhof, J. G. M., et al. Self-medication with Antimicrobial Drugs in Europe. Emerg Infect Dis. 2006; 12: 452–459. doi: 10.3201/eid1203.050992
9. Williams, P. C. M., Isaacs, D., and Berkley, J. A. Antimicrobial resistance among children in sub-Saharan Africa. Lancet Infect Dis. 2018;18: e33–e44. doi:10.1016/S1473-3099(17)30467-X
10. Smith, R., and Coast, J. The true cost of antimicrobial resistance. BMJ. 2013; 346: f1493. doi: 10.1136/bmj.f1493.
11. Shrestha, P., Cooper, B. S., Coast, J., et al. Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. Antimicrob Resist Infect Control. 2018; 7: 98. doi: 10.1186/s13756-018-0384-3
12. Walter, J., Noll, I., Feig, M., et al. Decline in the proportion of methicillin resistance among *Staphylococcus aureus* isolates from non-invasive samples and in outpatient settings, and changes in the co-resistance profiles: an analysis of data collected within the Antimicrobial Resistance Surveillance Network, Germany 2010 to 2015. BMC Infect Dis. 2017; 17: 169. doi: 10.1186/s12879-017-2271-6
13. Center for Disease Dynamics, Economics and Policy. State of the World's Antibiotics, 2015.
14. Abubakar, U., and Sulaiman, S. A. S. Prevalence, trend and antimicrobial susceptibility of Methicillin Resistant *Staphylococcus aureus* in Nigeria: a systematic review. J Infect Public Health. 2018; 11: 763–770. doi:10.1016/j.jiph.2018.05.013.
15. Joshi, S. Hospital antibiogram: a necessity. Indian J Med Microbiol. 2010; 28: 277–280. doi: 10.4103/0255-0857.71802.
16. CLSI. M100Ed33 | Performance Standards for Antimicrobial Susceptibility Testing, 33rd Edition. Clinical and Laboratory Standards Institute. https://clsi.org/standards/products/microbiology/documents/m100/ [Accessed Feb 20, 2024]
17. Manga, M. M., Ibrahim, M., Isaac, E. W., et al. Antibiogram of *Pseudomonas* species: an important tool to combat antibiotic resistance for patient safety in Gombe, Nigeria. Afr J Clin Exp Microbiol. 2021; 22: 279–283. doi:10.4314/ajcem.v22i2.21
18. Ali, F., Allu, M., and Assafi, M. The frequency of the pathogenic bacteria isolated from different clinical samples in Duhok, Iraq. 2020. https://doi/10.4108/eai.28-6-2020.2298236.
19. Dakorah, M. P., Agyare, E., Acolatse, J. E. E., et al. Utilising cumulative antibiogram data to enhance antibiotic stewardship capacity in the Cape Coast Teaching Hospital, Ghana. Antimicrob Resist Infect Control. 2022; 11: 122. doi: 10.1186/s13756-022-01160-5.
20. Santos, M., Mariz, M., Tiago, I., et al. A review on urinary tract infections diagnostic methods: Laboratory-based and point-of-care approaches. J Pharm Biomed Anal. 2022; 219: 114889. doi: 10.1016/j.jpba.2022.114889
21. Chen, C-J., and Huang, Y-C. New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect Dis. 2014; 20: 605–623. doi: 10.1111/1469-0691.12705.
22. Ezeh, C. K., Eze, C. N., Dibua, M. E. U., et al. A meta-analysis on the prevalence of resistance of *Staphylococcus aureus* to different antibiotics in Nigeria. Antimicrob Resist Infect Control. 2023; 12: 40. doi: 10.1186/s13756-023-01243-x
23. Manga, M. M., Michael, G. O., Julde, A. A., et al. Phenotypic Detection of Methicillin and Inducible Clindamycin Resistant *Staphylococcus aureus*: An Effort to Combat Antimicrobial Resistance and

Improve Patient Safety in Gombe, Nigeria. Ann Basic Med Sci. 2021; 2: 52–56. doi: 10.51658/abms.202121.3

24. Rağbetli, C., Parlak, M., Bayram, Y., et al. Evaluation of Antimicrobial Resistance in Staphylococcus aureus Isolates by Years. Interdiscip Perspect Infect Dis. 2016; 2016: 9171395. doi: 10.1155/2016/9171395
25. Achek, R., Hotzel, H., Cantekin, Z., et al. Emerging of antimicrobial resistance in staphylococci isolated from clinical and food samples in Algeria. BMC Res Notes. 2018; 11: 663. doi: 10.1186/s13104-018-3762-2.
26. Oche, D. A., Oyegoke, P. I., Akpudo, M. O., et al. Antibiotics Resistance Pattern of *Staphylococcus aureus* Isolated from In-patients of an Orthopaedic Hospital in North-Western Nigeria. Int J Epidemiol Res. 2021; 8: 160–166. doi: 10.34172/ijer.2021.29
27. Ariom, T., Iroha, I., Moses, I., et al. Detection and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* from clinical and community samples in Abakaliki, Ebonyi State, Nigeria. Afr Health Sci. 2019; 19: 2026–2035. doi: 10.4314/ahs.v19i2.26
28. Kengne, M., Fotsing, O., Ndomgue, T., et al. Antibiotic susceptibility patterns of *Staphylococcus aureus* strains isolated at the Yaounde Central Hospital, Cameroon: a retro prospective study. Pan Afr Med J. 2019; 32: 103. doi: 10.11604/pamj.2019.32.103.15743
29. Naimi, H. M., Rasekh, H., Noori, A. Z., et al. Determination of antimicrobial susceptibility patterns in *Staphylococcus aureus* strains recovered from patients at two main health facilities in Kabul, Afghanistan. BMC Infect Dis. 2017; 17: 737. doi: 10.1186/s12879-017-2844-4.
30. Seo, Y., and Lee, G. Antimicrobial Resistance Pattern in *Enterococcus faecalis* Strains Isolated from Expressed Prostatic Secretions of Patients with Chronic Bacterial Prostatitis. Korean J Urol. 2013; 54:477–481.doi:10.4111/kju.2013.54.7.477
31. Olawale, A. K., Salako, R. J., Olawale, A. O., and Famurewa. O. Antibiotic-Resistant *Enterococcus faecalis* Isolated from Food Canteens in Osun State, Nigeria. Microbiol Res J Int 2015; 6(4):196-206 doi: 10.9734/bmrj/2015/7348
32. Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., et al. High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. Germs. 2020; 10: 310–321. doi: 10.18683/germs.2020.1223
33. Agyepong, N., Govinden, U., Owusu-Ofori, A., et al. Multidrug-resistant gram-negative bacterial infections in a teaching hospital in Ghana. Antimicrob Resist Infect Contr. 2018; 7: 37. doi: 10.1186/s13756-018-0324-2
34. Lin, W-P., Wang, J-T., Chang, S-C., et al. The Antimicrobial Susceptibility of Klebsiella pneumoniae from Community Settings in Taiwan, a Trend Analysis. Sci Rep. 2016;6:36280. doi: 10.1038/srep36280.
35. Tansarli, G. S., Athanasiou, S., and Falagas, M. E. Evaluation of Antimicrobial Susceptibility of Enterobacteriaceae Causing Urinary Tract Infections in Africa. Antimicrob Agents Chemother. 2013; 57: 3628–3639. doi: 10.1128/AAC.00359-13
36. Wutayd, O. A., Nafeesah, A. A., Adam, I., et al. The antibiotic susceptibility patterns of uropathogens isolated in Qassim, Saudi Arabia. J Infect Dev Ctries. 2018;12:946–952.doi:10.3855/jidc.10553.
37. Ahmad, S., Alotaibi, M., and Mohmmed, S. Antibiotic Sensitivity Pattern of Clinical Isolates of *Pseudomonas aeruginosa* at a Tertiary Care Hospital in Saudi Arabia. Dhaka Univ J Pharm Sci. 2020; 19: 77–82. https://doi.org/10.3329/dujps.v19i1.47821
38. Yusuf, E., Van Herendael, B., Verbrugghe, W., et al. Emergence of antimicrobial resistance to *Pseudomonas aeruginosa* in the intensive care unit: Association with the duration of antibiotic exposure and mode of administration. Ann Intensive Care. 2017; 7: 72. doi:10.1186/s13613-017-0296-z.
39. Manga, M. M., Ibrahim, M., Hassan, U. M., et al. Empirical antibiotherapy as a potential driver of antibiotic resistance: observations from a point prevalence survey of antibiotic consumption and resistance in Gombe, Nigeria. Afr J Clin Exper Microbiol. 2021; 22: 273–278. doi:10.4314/ajcem.v22i2.20
40. Manga, M. M., Mohammed, Y., Suleiman, S., et al. Antibiotic prescribing habits among primary healthcare workers in Northern Nigeria: a concern for patient safety in the era of global antimicrobial resistance. PAMJ-One Health. 2021; 5: 19 doi:10.11604/pamj-oh.2021.5.19.30847

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Original Article **Open Access**

Bacterial etiology of spreading odontogenic infection in southwest Nigeria using the 16S rRNA next generation sequencing technique

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Abstract:

Background: Genomics surveillance and characterization of pathogens have enabled prompt and accurate diagnosis, for improved management and control of infectious diseases. This study aimed to identify bacteria associated with spreading odontogenic infections (SOIs) among patients visiting the Dental Center of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria, by sequencing 16S rRNA gene of the bacteria.

Methodology: This was a cross-sectional study of 15 participants with clinically confirmed SOIs. Pus samples were obtained from the participants and stored at -80^{0} C until ready for analysis. DNA was extracted from the pus samples using the Quick-DNA^{TM} Miniprep Plus Kit. Polymerase chain reaction (PCR) was used to amplify the V1-V9 regions of the 16S rRNA gene. Successfully amplified samples were cleaned up and next generation sequencing (NGS) technique was used to sequence the bacterial 16S rRNA gene. Sequence data were analyzed using Geneious Prime version 2021.2.2 which used Ribosomal Database Project Tools to assign genus and higher-level taxonomy.

Results: Multiple genera of bacteria were detected in individual sample. The detected and identified bacteria belonged to *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Proteobacteria*, *Tenericutes* and *Spirochaetes* phyla. Detected predominant bacterial genera were *Streptococcus, Prevotella*, *Peptostreptococcus, Parvimonas* and *Porphyromonas*. Some novel bacteria identified include *Legionella*, *Taonella*, *Ferrovibro*, *Holdemania*, and *Limnobacter*.

Conclusion: Bacteria detected in this study include previously reported bacteria associated with SOIs and novel bacteria, with preponderance of anaerobes.

Keywords: Spreading odontogenic infection; 16S rRNA; Next Generation Sequencing (NGS); Oral microbiota

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Étiologie bactérienne de la propagation de l'infection odontogène dans le sud-ouest du Nigeria à l'aide de la technique de séquençage de nouvelle génération de l'ARNr 16S

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Resumé:

Contexte: La surveillance génomique et la caractérisation des agents pathogènes ont permis d'établir un diagnostic rapide et précis, afin d'améliorer la prise en charge et le contrôle des maladies infectieuses. Cette étude visait à identifier les bactéries associées à la propagation des infections odontogènes (SOI) chez les patients visitant le Centre dentaire du Complexe des hôpitaux universitaires Obafemi Awolowo (OAUTHC), à Ile-Ife, au Nigeria, en séquençant le gène de l'ARNr 16S de la bactérie.
Méthodologie: Il s'agissait d'une étude transversale portant sur 15 participants présentant des troubles de l'information cliniquement confirmés. Des échantillons de pus ont été prélevés chez des participants atteints de SOI et stockés à -80^{0}C jusqu'à ce qu'ils soient prêts à être analysés. L'ADN a été extrait d'échantillons de pus à l'aide du kit Quick-DNA™ Miniprep Plus. La réaction en chaîne par polymérase (PCR) a été utilisée pour amplifier les régions V1-V9 du gène de l'ARNr 16S. Les échantillons ont été amplifiés et nettoyés avec succès. Une technique de séquençage de nouvelle génération a été utilisée pour séquencer le gène bactérien de l'ARNr 16S. Les données de séquence ont été analysées à l'aide de la version 2021.2.2 de Geneious Prime, qui a utilisé les outils de projet de base de données ribosomique pour attribuer le genre et la taxonomie de niveau supérieur.
Résultats: Plusieurs genres de bactéries ont été détectés dans un échantillon individuel. Les bactéries détectées et identifiées appartenaient aux embranchements *Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria, Tenericutes* et *Spirochaetes*. Les genres bactériens prédominants détectés étaient *Streptococcus, Prevotella, Peptostreptococcus, Parvimonas* et *Porphyromonas*. Parmi les nouvelles bactéries identifiées, citons Legionella, Taonella, Ferrovibro, Holdemania et Limnobacter.
Conclusion: Les bactéries détectées comprennent des organismes bactériens précédemment signalés associés à la SOI et de nouvelles bactéries avec une prépondérance d'anaérobies.

Mots-clés: Propagation de l'infection odontogène; séquençage du gène de l'ARN 16S; Séquençage de nouvelle génération (NGS); Microbiote buccal

Introduction:

Spreading odontogenic infections (SOIs) are severe form of odontogenic infections that have extended to involve the deeper tissues of the head and neck region typically through the lateral surface of the alveolar bone (1). Poorly treated and untreated dentoalveolar infections will almost always progress into SOIs. It is a mixed infection involving mostly bacteria of the normal oral microbiota that become opportunistic pathogens. Common clinical presentations are swelling and pain over the anatomic space involved in the head and neck region, restricted mouth opening, dysphagia and fever (2).

Late presentation and/or delayed appropriate interventions often result in poor outcomes such as airway obstruction/respiratory distress, prolonged hospitalization with/without intensive care admission, orbital cellulitis, cervicofacial necrotizing fasciitis, cavernous sinus thrombosis, brain abscess, sepsis and death. Systemic co-morbidities such as diabetes mellitus, chronic liver disease, malnutrition and any condition that impairs host immunity are associated with complications and prolonged hospital stay (2-4).

Traditionally, the putative bacterial pathogens of SOIs are identified by culture of clinical specimens (mostly pus) and usually followed by antibiotics sensitivity testing in microbiology laboratory. This approach is time consuming, especially when fastidious, slow-growing oral pathogens are involved, and can only detect organisms that can be cultured. Even with recent advances in culture techniques, about one third of oral bacteria are still uncultured (5).

Molecular biology techniques such as polymerase chain reaction, cloning and sequence analysis of bacterial 16S rRNA genes have been used to identify some bacteria associated with SOIs and detect antibiotic-resistant strains which could enhance clinical outcomes by facilitating development and implementation of rational surgical and supportive treatment protocols (6,7).

Böttger et al., (8) sequenced the 16S rRNA gene by next generation sequencing (NGS) platform to identify bacterial organisms in the pus samples of Caucasian patients with severe odontogenic infections. The study concluded that 16S rRNA gene metagenomics detected more bacteria than the conventional culture dependent method and also confirmed bacteria in previous culture-negative samples. There is paucity of published report on molecular identification of bacteria associated with SOIs in Nigeria. Considering the high diagnostic yield of 16S rRNA gene sequencing by high throughput NGS platform and its likely clinical benefits on the overall management of patients with SOIs, this study aimed to identify bacteria associated with SOI in Nigerian patients by sequencing bacterial 16S rRNA gene using the NGS technique.

Materials and method:

Study design and participants:

This was a cross-sectional study of a total of 15 participants with clinically confirmed spreading odontogenic infections (SOIs) who were enrolled into the study, with the aim of identifying bacteria associated with SOI through 16S rRNA gene sequencing.

Ethical consideration:

The study was approved by the Ethics

and Research Committee (Protocol no: ERC/ 2021/05/12) of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife Osun State, Nigeria. Written informed consents were obtained from all confirmed patients with SOI visiting the Dental Center of OAUTHC, Ile-Ife before enrollment into the study. All procedures in the study were in compliance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Sample collection:

Pus was aseptically collected by sterile needle aspiration using disposable 5ml syringe with a 21G needle through the povidone iodine prepared mucosa (for intraoral swellings) or skin overlying facial and upper neck swellings. Sampling site was determined by swelling with maximum fluctuance intraorally and extra-orally. The pus samples were transferred to a labelled Eppendorf vial containing 1ml of sterile phosphate buffered saline (PBS) and transported to the African Center of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University Ede, Osun State Nigeria on ice packs. All samples were stored at –80°C until molecular assay was conducted at the ACEGID laboratory.

DNA extraction from pus samples:

DNA was extracted using Quick-DNA™ Miniprep Plus Kit (Zymo Research Corporation) according to the manufacturer's instructions. A final volume of 50 µL of the DNA was eluted into a clean labelled microcentrifuge tube and store at -20°C until use for the analysis.

Polymerase chain reaction of 16S rRNA gene:

The universal primers used for PCR targeted the conserved V1-V9 regions of the 16S rRNA gene were 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-AAG GAG GTG ATC CAG CCG CA-3') forward and reverse primers respectively. This amplification was done in a final reaction volume of 20 µL using PuReTaq™ Ready-To-Go PCR Beads that were reconstituted with 12.5 µL of double distilled water, 1.25 µL each of the forward and reverse primers (each primer at a concentration of 0.1µM) and 5 µL of extracted DNA sample (at concentration of 5 ng/µL). The amplification was done in the Eppendorf Mastercycler PRO S 6325 Thermal Cycler set at appropriate cycling conditions as previously described (7).

Sequencing of 16S rRNA gene amplicons:

Samples of 9 patients with severe SOI with or without concomitant systemic disease were selected for sequencing of the 16S rRNA gene. The amplified products were cleaned up using QIAquick® PCR & Gel Cleanup Kit, and libraries of the amplicons were prepared according to protocol for PulseNet Nextera XT library prep and run setup for the Illumina MiSeq (9). The NGS technique was used to sequence the bacterial 16S rRNA gene using the Illumina MiSeq (Illumina, San Diego, CA) sequencing system (9). The sequence data for this study were submitted to the SRA database (accession number: PRJNA926479) and are available at: https://www.ncbi.nlm.nih.gov/sra/PRJNA926479

Bioinformatics analysis of the sequence data:

Illumina MiSeq sequence data were uploaded into the Geneious Prime version 2021.2.2 (https://www.geneious.com) (10). Data were cleaned by trimming, merging and length filtering to eliminate PCR and sequencing errors, and chimeric sequences before analysis by a web-based 16S Biodiversity tool. Merged sequence reads were clustered into operational taxonomic units (OTUs) at the 98% similarity cut off point. The platform uses Ribosomal Database Project (RDP) Tools (11) version 2.12 which assigned taxonomy (from domain to genus) and bootstrap confidence-estimate to each sequence by comparing them to sequences on the 16S rRNA database.

The interactive krona chart of bacterial diversity based on the assigned bacterial taxonomy was generated by Krona version 2.0 (12) for each sequenced sample. Detected bacterial genera were compared with the expanded Human Oral Microbiome Database (13). This was done to ascertain if these organisms are previously reported resident oral microbiota or exogeneous pathogenic organisms.

Statistical analysis:

Bacterial diversity in the samples was determined by alpha diversity indices such as Shannon and Chaol 1 while between sample diversity was assessed by Bray-Curtis similarity index (14,15).

Results:

Demographics and clinical profile of study participants:

Overall, 15 participants with clinically confirmed SOIs were enrolled into the study. The mean age of the study participants was 51.8 years (range: 27-84 years). Eight of the participants were females. Twelve participants (80.0%) presented with no underlying co-morbidity while three participants had hypertensive heart disease and diabetes mellitus (Table 1).

Results of PCR, 16S rRNA gene sequencing and Bioinformatics analysis:

The PCR amplification of the V1-V9 region of the bacterial 16S rRNA gene was successful in 14 (93.3%) of the 15 pus samples. Based on the clinical presentations (severity of infection and presence of systemic dis-

ease) of the participants, 9 (64.3%) of the 14 amplified samples were successfully sequenced using Illumina MiSeq sequencing platform. The total number of sequence reads was 23,925,665 after trimming, merging and length filtering, with a median read of 2,545,659 (Table 2).

Table 1: Demographics and clinical profiles of study participants with spreading odontogenic infections

Patient code	Gender	Age (years)	Systemic disease	Sampling site
01	Female	60	Hypertensive heart disease	Maxillary vestibule
03	Male	38	Nil	Submandibular space
04	Male	45	Nil	Submandibular space
05	Male	31	Nil	Buccal space
06	Male	65	Nil	Submandibular space
07	Male	38	Nil	Buccal space
11	Male	27	Nil	Maxillary vestibule
12	Female	80	Hypertensive heart disease	Maxillary vestibule
15	Female	84	Diabetes mellitus	Maxillary vestibule
02	Female	45	Nil	Mandibular vestibule
08	Female	58	Nil	Submandibular space
09	Female	65	Nil	Maxillary vestibule
10	Female	35	Nil	Mandibular vestibule
13	Female	57	Nil	Maxillary vestibule
14	Male	50	Nil	Maxillary vestibule

Table 2: Valid 16S rRNA gene sequence reads and operational taxonomic units (OTUs)

Patient code	Sample ID	Total sequenced read	Total OUT
01	OIF1	4,290,898	43,090
06	OIF2	4,673,600	7,852
07	OIF3	4,270,008	53,231
05	OIF4	2,416,339	1,602
11	OIF5	4,676,708	12,552
04	OIF6	1,281,138	86,286
12	OIF7	2,545,659	13,677
03	OIF8	2,004,437	1,044,797
15	OIF9	1,766,878	1,025,678

ID: Identification code; OTU: Operational Taxonomic Unit

The abundance of detected operational taxonomic units (OTUs) is represented by radial slices of the Krona charts and the hue (red to green) highlights the average confidence value for each segment. Low confidence is shown as red and it increases to green with a higher confidence for taxonomic classification.

All the nine samples revealed the presence of a wide range of bacteria as shown in the Krona charts for samples OIF1 (Fig 1) and OIF2 to OIF9 (Supplementary Figs 1-8) (https://afrjcem.org/supplementary-materials/).

Detected bacteria and diversity analysis:

The average number of bacteria found in the 9 sequenced samples was 269,209 (Range: 585-1,154,363). Detected bacteria in the 9 samples belonged to at least 7 phyla, namely *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Proteobacteria*, *Tenericutes* and *Spirochaetes* phyla.

The different abundant bacterial genera detected in the samples are presented in Fig 2 with *Streptococcus, Prevotella*, *Peptostreptococcus Parvimonas* and *Porphyromonas* genera as the most predominant bacteria in the pus samples. Expectedly, there were more anaerobic than aerobic bacteria in the pus samples.

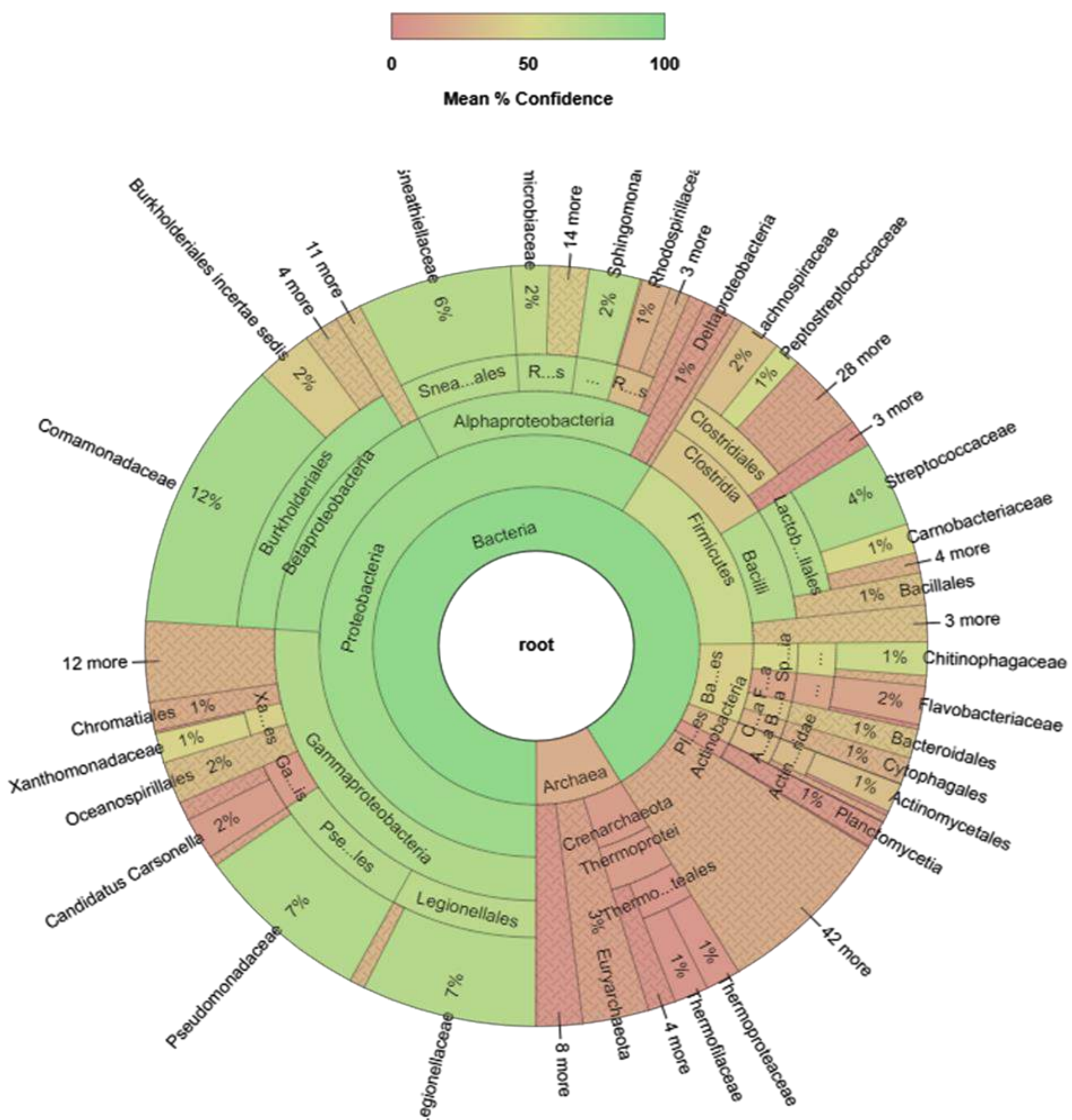

Fig 1: Krona chart of sample OIF1

Full names of truncated legends/labels: Sneathiellaceae, Hyphomicrobiaceae, Sphingomonadaceae, Rhodospirallaceae, Legionellaceae

The Shannon diversity index was highest in sample OIF8 (2.183), followed by OIF1 (2.097) and lowest in OIF5 (1.770). The values of Chao1 index revealed that diversity was highest in OIF1 (16.0), followed by OIF2 and OIF8, and lowest in OIF5 and OIF9 (Table 3). The mean alpha diversity indices of intraoral samples were 1.9495 (Shannon index) and 11.125 (Chao 1 index). Conversely, mean alpha diversity indices of extraoral samples were 2.0348 (Shannon index) and 10.55 (Chao 1 index).

For beta diversity, bacterial genera composition was similar between OIF4 and OIF9 (Bray-Curtis similarity index of 0.79), followed by OIF5 and OIF6, then OIF1 and OIF7 (Fig 3). Pairwise comparison of beta diversity indices of extraoral with intraoral samples showed 100% similarity between six pairs of intraoral and extraoral samples (Supplementary Table 1). Supplementary Table 2 showed 15 bacterial genera detected but were not known to be associated with SOIs nor part of resident oral microbiota when compared with publicly available oral microbiome database (https://afrjcem.org/supplementary-materials/).

Table 3: Alpha diversity indices of individual sample microbiota

Sample ID	Sampling site	Shannon index	Chao 1 index
OIF1	Intraoral/Maxillary vestibule	2.097	16.00
OIF2	Extraoral/Submandibular space	2.047	11.00
OIF3	Extraoral/Buccal space	1.903	10.50
OIF4	Extraoral/Buccal space	1.927	10.25
OIF5	Intraoral/Maxillary vestibule	1.77	9.00
OIF6	Extraoral/Submandibular space	2.064	10.00
OIF7	Intraoral/Maxillary vestibule	2.075	10.50
OIF8	Extraoral/Submandibular space	2.183	11.00
OIF9	Intraoral/Maxillary vestibule	1.856	9.00

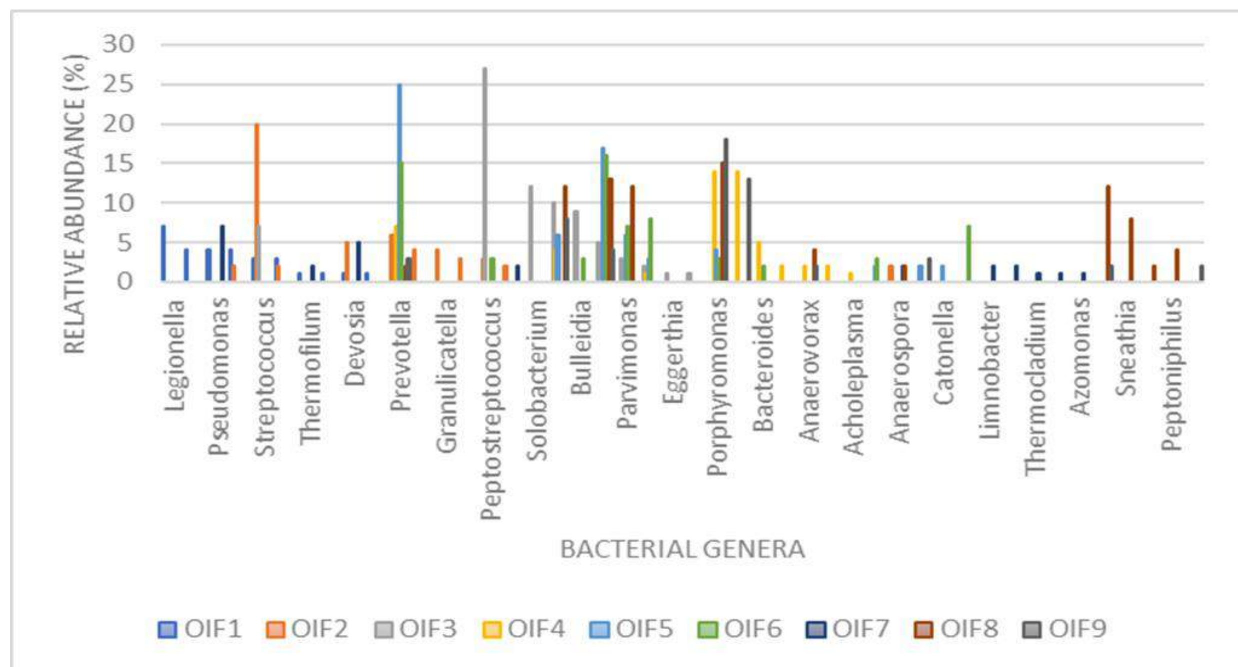

Fig 2: Clustered bar chart showing different abundant bacterial genera in pus samples
Bacterial genera with less than 1% abundance were excluded

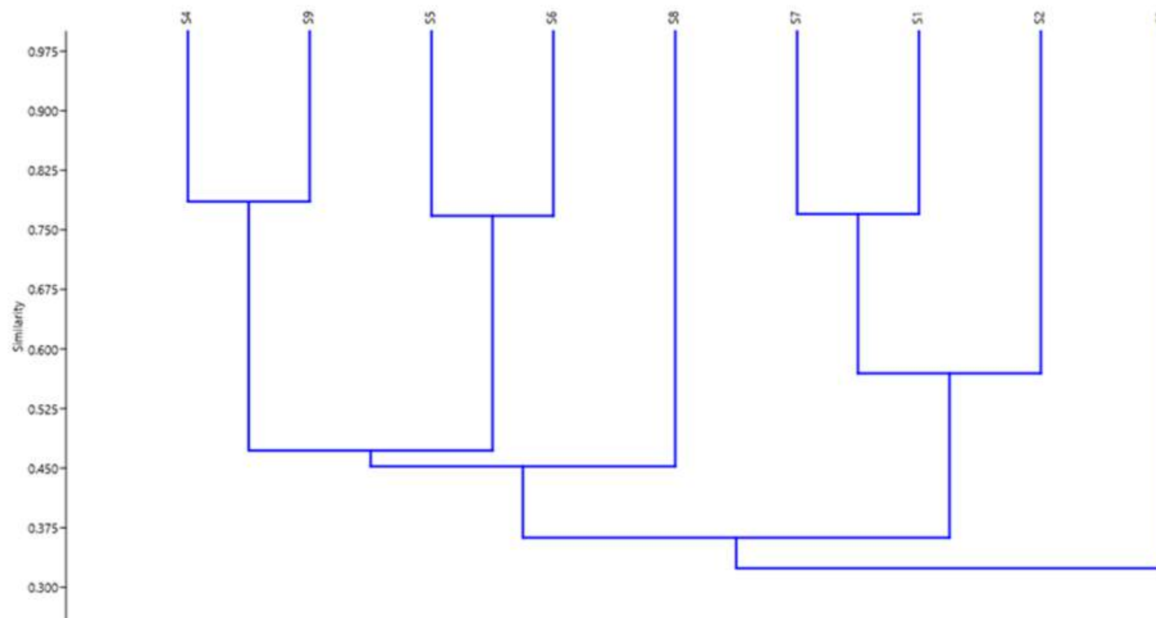

Fig 3: Dendrogram showing the Bray-Curtis similarity of bacterial genera in nine pus samples*
*S1=OIF1; S2=OIF2; S3=OIF3; S4=OIF4; S5=OIF5; S6=OIF6; S7=OIF7; S8=OIF8; S9=OIF9

Discussion:

This study was undertaken to identify bacteria associated with SOIs in Southwest Nigeria by sequencing 16S rRNA gene in order to comprehensively determine the bacterial etiology of SOI in the study population. The spectrum of bacteria detected and identified in this study belonged to seven phyla (Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria and Spirochaetes) to which previously reported bacteria species associated with odontogenic abscesses are classified (16). This shows that the phylum level biodiversity of this study is broader than similar studies on SOIs that used lower throughput molecular methods such as Sanger sequencing and nested PCR.

Furthermore, *Streptococcus, Prevotella*, *Peptostreptococcus, Parvimonas* and *Porphyromonas* genera were found to be the most predominant bacteria detected in the pus samples. This is similar to the reports of Bottger and colleagues (8) in Germany where *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Veillonella* and *Parvimonas* genera were the most prevalent organisms after 16S rRNA gene sequencing in 48 patients with severe odontogenic infection. This trend is typical of the microbial pathogenesis of odontogenic infections. Specifically, *Prevotella intermedia*, *Prevotella nigrescens*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum* have been reported as the most important pathogenic anaerobes implicated in the pathogenesis of orofacial odontogenic infections because of their virulence factors and synergistic relationship with other bacteria (17).

Bacteriology of SOIs involved predominantly anaerobes, with aerobic bacteria in the early phase of infections. Aerobic bacteria serve as initiators of the infectious process, preparing the local environment for anaerobic bacterial invasion as the local tissue condition turns to a more hypoxic state that encourages anaerobic bacterial growth but inhibits aerobic bacterial growth (18,19). This microbial succession was validated in the present study as anaerobes were prominent among the bacterial genera detected and identified in all the samples. Since the bacterial profile of SOI is polymicrobial (with preponderance of anaerobes), the first line antibiotic therapy should address these organisms with immediate surgical intervention to drain abscess and/or decompress cellulitis.

Accurate identification of microorganisms implicated in SOI is critical in patients' timely and efficient management. This guides the prescription of antibiotic therapy as a potent adjunct to the surgical management of SOI. Appropriate non-surgical treatment along with antibiotics therapy is particularly important in immunocompromised and medically compromised patients with SOI (20). The treatment goal is to prevent or minimize rapid spread of infection to contiguous areas and septic complication (21).

Plethora of bacterial organisms were identified at the genus level in this study. This is enormously diverse and overwhelming in terms of counts compared to the limited number of identified bacterial species associated with SOIs as reported by Flynn et al., (6) and Walia et al., (21) who used Sanger sequencing technique and culture method respectively. Diagnostic yield of 16S rRNA gene sequencing by high throughput NGS technologies have

significantly expanded the knowledge of the bacterial diversity associated with predominantly bacterial odontogenic infections (22).

Analysis of bacterial diversity based on the metrics of Shannon and Chao1 indices showed that pus samples OIF1 and OIF8 have high microbial diversity than other samples. Intraoral samples had more diversity (Chao 1 index) than extraoral samples. Conversely, intraoral samples had lower mean Shannon index, that measured species richness and evenness. With regards to beta diversity, there were more than 70% similarity in the bacterial composition of six out of the nine samples. This is probably because most odontogenic infections are caused by opportunistic resident oral microbiota (18).

There is dearth of normal oral 16S rRNA derived microbiome data in Nigeria and indeed Africa (23,24). Accordingly, we compared our data with the expanded Human Oral Microbiome Database (13). Following this, there were 15 bacterial genera which to the best of our knowledge, have not been previously associated with SOIs nor are normal oral microbiota. The detection of these novel organisms may be attributed to the sensitivity and wider coverage (deep sequencing of 16S rRNA gene) of the molecular technique used in this study (22). Furthermore, race and diets of study participants could be responsible for this finding as reported by Yang et al., (25).

Apart from the small sample size, this study is also limited by the fact that there was no oral microbiome analysis from individuals with no SOIs as comparative or control group. This would have enabled us to objectively determine whether detected bacteria were part of the resident oral microbiota in the studied population or exogenous bacteria. However, majority of the detected bacteria in this study have been reported to be opportunistic pathogens implicated in SOIs and other odontogenic infections (6-8).

In conclusion, our study provides important information on the bacteriology of SOIs in the study population using 16S rRNA gene NGS technique. We recommend further study with adequate sample size, and age and gender matched control group to validate our findings and assign taxonomy to identified organisms up to the species level.

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Contributions of authors:

BAF and OAF were involved in the study conceptualization and design; BAF, SBA and FOO were involved with participants' recruitment and clinical sample acquisition; BAF, JNU and PE were involved in molecular experiment and investigation; IBO and BAF were involved with analysis and interpretation of sequence data; CTH was responsible for funding acquisition; OAF and CTH were involved with project administration; OAF was responsible for project supervision; BAF wrote the original draft; BAF, OAF and IBO reviewed and edited the manuscript. All authors read and approved the manuscript submitted for publication.

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Conflict of interest:

No competing interest is declared.

Data availability:

The dataset of this study is available from the corresponding author on reasonable request. The sequence data for this study were submitted to the SRA database (accession number: PRJNA926479) and are available at https://www.ncbi.nlm.nih.gov/sra/PRJNA926479

References:

1. Lopez-Gonzalez, E. Vitales-Noyola, M., González-Amaro, A. M., et al. Aerobic and anaerobic microorganisms and antibiotic sensitivity of odontogenic maxillofacial infections. Odontology. 2019; 107:409-417. https://doi.org/10.1007/s10266-019-00414-w
2. Adeosun, P. O., Fatusi, O. A., and Adedeji, T. A. Assessment of severity of illness and monitoring response to treatment of odontogenic space infection using serum prealbumin. J Maxillofac Oral Surg. 2019; 18: 106–111. https://doi.org/10.1007/s12663-018-1105-4
3. Weise, H., Naros, A., Weise, C., Reinert, S., and Hoefert, S. Severe odontogenic infections with septic progress – a constant and increasing challenge: a retrospective analysis. BMC Oral Health. 2019; 19:173. https://doi.org/10.1186/s12903-019-0866-6
4. Bali, R.K., Sharma, P., Gaba, S., Kaur, A., and Ghanghas P. A review of complications of odontogenic infections. Natl J Maxillofac Surg. 2015; 6:136-143. https://doi.org/10.4103/0975-5950.183867
5. Wade, W., Thompson, H., Rybalka, A., Vartoukian, S. Uncultured Members of the Oral Microbiome. J Calif Dent Assoc. 2016; 44: 447-456.
6. Flynn, T. R., Paster, B. J., Stokes, L. N., Susarla, S. M., and Shanti, R. M. Molecular methods for diagnosis of odontogenic infections. J Oral Maxillofac Surg. 2012; 70:1854-1859. https://doi.org/10.1016/j.joms.2011.09.009
7. Riggio, M. P., Aga, H., Murray, C. A., et al. Identification of bacteria associated with spreading odontogenic infections by 16S rRNA gene sequencing. Oral Surg Oral Med Oral Pathol Oral

Radiol Endod. 2007; 103:610-617. https://doi.org/10.1016/j.tripleo.2006.08.009
8. Böttger, S., Zechel-Gran, S., Schmermund, D., et al. Clinical relevance of the microbiome in odontogenic abscesses. Biology (Basel). 2021; 10: 916; doi: 10.3390/biology10090916
9. Centers for Disease Control and Prevention. Laboratory standard operating procedure for PulseNet Nextera XT Library Prep and Run Setup for the Illlumina MiSeq. Code PNL32. (2015). https://www.cdc.gov/pulsenet/pdf/pnl32-miseq-nextera-xt.pdf (Accessed February 1st, 2016)
10. Kearse, M., Moir, R., Wilson, A., et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199
11. Cole, J. R., Wang, Q., Fish, J. A., *et al*. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucl Acids Res. 2014; 42: D633-D642. https://doi.org/10.1093/nar/gkt1244
12. Ondov, B. D., Bergman, N. H., Phillippy, A. M. Interactive metagenomic visualization in a Web browser. BMC Bioinform. 2011; 12: 385. https://doi.org/10.1186/1471-2105-12-385
13. Escapa, F. I., Huang, Y., Chen, T., et al. Construction of habitat-specific training sets to achieve species-level assignment in 16S rRNA gene datasets. Microbiome 2020; 8: 65. https://doi.org/10.1186/s40168-020-00841-w
14. Kim, B. R., Shin, J., Guevarra, R., et al. Deciphering diversity indices for a better understanding of microbial communities. J Microbiol Biotechnol. 2012; 27: 2089–2093. https://doi.org/10.4014/jmb.1709.09027
15. Ricotta, C., and Podani, J. On some properties of the Bray-Curtis dissimilarity and their ecological meaning. Ecol Complex. 2017; 31:201–205. https://doi.org/10.1016/j.ecocom.2017.07.003
16. Siqueira, J. F., and Rocas, I. N. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev. 2013; 26:255–273. https://doi.org/10.1128/cmr.00082-12
17. Stefanopoulos, P. K., and Kolokotronis, A. E. The clinical significance of anaerobic bacteria in acute orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 98:398-408. https://doi.org/10.1016/j.tripleo.2004.01.016
18. Ogle, O. E. Odontogenic infections. Dent Clin N Am. 2017; 61: 235–252. https://doi.org/10.1016/j.cden.2016.11.004
19. Zirk, M., Buller, J., Goeddertz, P. et al. Empiric systemic antibiotics for hospitalized patients with severe odontogenic infections. J Craniomaxillofac Surg. 2016; 44:1081-1088. https://doi.org/10.1016/j.jcms.2016.05.019
20. Flynn, T. R., Shanti, R. M., Levi, M. H., Adamo, A. K., Kraut, R. A., and Trieger, N. Severe odontogenic infections, part 1: prospective report. J Oral Maxillofac Surg. 2006; 64:1093–1103. https://doi.org/10.1016/j.joms.2006.03.015
21. Walia, I. S., Borle, R. M., Mehendiratta, D., and Yadav, A. O. Microbiology and antibiotic sensitivity of head and neck space infections of odontogenic origin. J. Maxillofac. Oral Surg. 2014; 13: 16–21. https://doi.org/10.1016/j.jcms.2017.07.013
22. Wade, W. G., and Prosdocimi, E. M. Profiling of Oral Bacterial Communities. J Dent Res. 2020; 99:621-629. https://doi.org/10.1177/0022034520914594
23. Anukam, K. C., and Agbakoba, N. R. A comparative study of the oral microbiome compositions of healthy postmenopausal, premenopausal, and prepubertal Nigerian females, using 16S rRNA metagenomics methods. Niger J Clin Pract. 2017; 20:1250-1258. https://doi.org/10.4103/njcp.njcp_32_17
24. Araújo, V., Fehn, A. M., Phiri, A., Wills, J., Rocha, J., and Gayà-Vidal, M. Oral microbiome homogeneity across diverse human groups from southern Africa: first results from southwestern Angola and Zimbabwe. BMC Microbiol. 2023; 23: 226. https://doi.org/10.1186/s12866-023-02970-2
25. Yang, Y., Zheng, W., Cai, Q., et al. Racial differences in the oral microbiome: data from low-income populations of African ancestry and European ancestry. mSystems. 2019; 4: e00639-19. doi:10.1128/mSystems.00639-19

Gawa et al. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (2): 144 - 150 https://www.afrjcem.org

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Prevalence and aetiological agents of childhood urinary tract infections at the University Teaching Hospital (CHU) of Bouaké, Côte d'Ivoire

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Abstract:

Background: Urinary tract infections (UTIs) are common in paediatric environment. The microbial ecology is often modified by over-prescription of antibiotics. The aim of this study was to determine the prevalence and etiological agents responsible for paediatric UTIs, with a view to improving care for children in Bouaké, Côte d'Ivoire.
Methodology: The study was carried out in the Paediatrics Department and the Microbiology Laboratory of Bouaké University Hospital Center over a 30-month period from June 2020 to December 2022. A total of 219 children were included and urine samples were collected. Bacterial strains were identified using conventional bacteriology techniques. Antibiotic susceptibility testing was carried out using the disk diffusion method, and interpretation was made according to CASFM/EUCAST recommendations for the current year.
Results: Among the 219 children included, 28 (12.8%) cases of UTIs were diagnosed and urinary tract anomalies in 21.4% of the UTI cases (6/28). The median age of the UTI cases was 96 months, females accounted for 60.7% (17/28) with a M/F of 0.65. Fever and urinary symptoms were reported by 47.0% and 17.3% respectively. Community acquired UTIs occurred in 25.0% (7/28), while hospital acquired UTIs occurred in 75.0% (21/28). *Escherichia coli* (60.7%) and *Klebsiella pneumoniae* (17.9%) were the most frequent bacteria isolated. Resistance rates to standard beta-lactam antibiotics ranged from 56.3% to 62.3%.
Conclusion: The epidemiology of paediatric urinary tract infections was dominated by *E. coli,* with high rates of resistance to standard antibiotics at Bouaké University Hospital Center.

Key words: Urinary tract infections, Paediatrics, Antibiotic resistance, Côte d'Ivoire

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Prévalence et agents étiologiques des infections urinaires de l'enfant au CHU de Bouaké, Côte d'Ivoire

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Résumé:

Contexte: Les infections urinaires (IU) sont fréquentes en milieu pédiatrique. L'écologie microbienne est souvent modifiée par la prescription abusive des antibiotiques. L'objectif de cette étude était de déterminer la prévalence et les agents étiologiques responsables d'IU pédiatrique pour une meilleure prise en charge des enfants à Bouaké.
Méthodologie: L'étude a été réalisée dans les services de Pédiatrie et le Laboratoire de Microbiologie du CHU de Bouaké durant une période 30 mois allant de Juin 2020 à Décembre 2022. Un total de 219 enfants a été inclus et des échantillons d'urines ont été prélevés. Les souches bactériennes ont été identifiées selon les techniques de

bactériologie conventionnelle. Le test de sensibilité des bactéries aux antibiotiques a été réalisé par la méthode de diffusion en milieu gélosé Mueller-Hinton et l'interprétation a été faite selon les recommandations CASFM/ EUCAST de l'année en cours.
Résultats: Parmi les 219 enfants inclus, 28 cas d'infections urinaires ont été diagnostiqués (12,8%) et les anomalies de l'appareil urinaire 21,4% (6/28). L'âge médian=96 mois. Les femmes représentaient 60,7% (17/28) avec une sex-ratio H/F=0,65. La fièvre et les signes d'appel urinaire étaient évoqués respectivement 47% et 17,3%. Par ailleurs, le taux d'infection urinaire d'origine communautaire était de 25,0% (7/28) contre 75,0% (21/28) en milieu hospitalier. *Escherichia coli* (60,7%), *Klebsiella pneumoniae* (17,9%) étaient les principales bactéries isolées. Les taux de résistance aux bêta lactamines usuels étaient variables de 56,3 à 62,3%.
Conclusion: l'épidémiologie des infections urinaires pédiatrique était dominé par *E. coli* avec des taux de résistances élevés vis-à-vis des antibiotiques usuels au CHU de Bouaké.

Mots-clés: Infections urinaires, Pédiatrie, Résistance aux antibiotiques, Côte d'Ivoire

Introduction:

After respiratory tract infections (RTI), urinary tract infections (UTIs) are the second most common clinical conditions for consultations and antibiotic prescriptions (1). They can affect the urinary tract at any age, but are a frequent source of infection in infants and young children in particular (2,3). There are around 150 million cases of UTIs every year worldwide, including almost 2 million in France, making it a major public health concern (4). In developed countries, it accounts for around 5% of hospital admissions in general paediatric wards (5). In Morocco, Zahir (6) reported a frequency of UTI of 30.2%.

In Burkina Faso, the frequency of UTI was 18.7% at the Centre Hospitalier Universitaire pédiatrique de Ouagadougou in 2012 (7). At the Paediatrics Department of Yopougon University Hospital, Côte d'Ivoire in 2014, the frequency of UTI was estimated to be 25%, and at Cocody University Hospital, UTI accounted for 18.9% of neonatal infections (8).

Urinary tract infection in children is responsible for serious complications such as arterial hypertension and chronic kidney disease (9) and therefore requires rapid and effective treatment. However, the urine cytobacteriological examination (ECBU), which confirms the presence of a UTI and identifies the bacteria responsible, is rarely carried out as part of the management of the infection due to the low socio-economic status of most patients (8). In this context, treatment is generally empirical, leading to frequent therapeutic failures with antibiotics prescribed as first-line treatment.

The increasing antibiotic resistance of bacteria involved in UTIs also limits the choice of antibiotics, hence the importance of bacteriological documentation of UTIs and the choice of appropriate antibiotic therapy (10). The aim of this study was to determine the prevalence and aetiological agents responsible for paediatric UTIs in order to improve management of children in Bouaké.

Materials and method:

Study setting, design and duration:

This was a descriptive cross-sectional study conducted in the Paediatrics Department and the Microbiology Laboratory of the University Hospital of Bouaké over a period of 30 months (June 2020 to December 2022). The study population was represented by children aged 0 to 15 years, regardless of sex, presenting with fever with or without urinary symptoms, or in whom urine cytobacteriological examination (ECBU) had been prescribed. Patients who had received antibiotic therapy for more than 48 hours prior to the ECBU were excluded.

Ethical considerations:

The Scientific Medical Department (DMS) of the University Hospital Centre (CHU) of Bouaké, acting as the Ethics Committee, approved the study. Informed consent was obtained from the children's parents or legal guardians.

Clinical sample collection:

Fresh urine was collected in a sterile container. In neonates and infants, samples were taken after rigorous asepsis of the perineal area using Urinicols. These devices were left in place for a maximum of 30 minutes until urination. In young and older children, urine was collected in sterile urine jars using the jet medium technique (11). The urine samples were transported to the laboratory immediately for bacteriological analysis

Bacteriological analysis:

The urine collected was analysed using conventional bacteriological techniques including macroscopic examination to assess the clarity of the urine and macroscopic haematuria, and direct microscopic examination after Gram staining. Cytological analysis (qualitative and quantitative) was performed to assess leukocyturia.

Urine samples were cultured on Eosin Methylene Blue (EMB) and Bromocresol Purple

(BCP) media and samples meeting the Kass positivity criteria (leucocyturia of > 10^4 leucocytes/ml and bacteriuria of >10^5CFU/ml) were identified for further processing.

Enterobacteriaceae were identified using conventional morphological and biochemical test scheme. Gram-positive cocci were tested for catalase and sub-cultured on Chapman and Bile Esculin Azoture (BEA) media, enabling staphylococci strains to be distinguished from enterococci. Pastorex Staph-Plus kits were used to distinguish *Staphylococcus aureus* from other staphylococci. The Novobiocin disc was used to identify *Staphylococcus saprophyticus*.

Antibiotic susceptibility testing and resistance detection:

Antibiotic susceptibility of each isolate was determined using the disk diffusion method on Mueller-Hinton agar. Inhibition diameters were interpreted as sensitive, intermediate or resistant according to CASFM/EUCAST criteria for the current year.

Enterobacteriaceae were tested for ex tended-spectrum beta-lactamase (ESBL) production using the double disk synergy test with a central disc of amoxicillin + clavulanic acid 30 mm from the cefotaxime, ceftriaxone and aztreonam discs. The presence of ESBL was noted in the presence of a 'champagne cork' appearance.

Statistical analysis of the data:

The variables studied were epidemiological (sex, age, patient origin), clinical and bacteriological data, and the rate of bacterial resistance to antibiotics. The data were analysed using EPI-INFO 7.2.2.6 software, and the Chi² test was used to compare qualitative variables with significance level set at $p<$ 0.05.

Results:

Prevalence of UTI:

During the study, urine samples were collected from 219 patients, who were hospitalized in paediatric department in 75.3% (165/219) and from the community (paediatric consultation) in 24.7% (54/219). The median age of the patients was 6 years, with a range of 1 day to 15 years. The 6-15 years age group was the most represented (50.7%), followed by the 2-5 years age group (28.8%) (Table 1).

The most frequent symptom for requesting ECBU was fever (47.0%), followed by urinary burning (17.3%) and dysuria (8.2%) (Table I). Antibiotic therapy had been initiated in 29.6% of patients and for at least 24 hours in 78.5% of them (Table 1). The antibiotics most frequently prescribed were ceftriaxone (41.5%) and amoxicillin-clavulanic acid (24.6%).

Table 1: Clinical and demographic characteristics of the study participants

Variable	Number (%)
Age group (years)	
0-2	45 (20.5)
2-5	63 (28.8)
6-15	111 (50.7)
Sex	
Male	134 (61.2)
Female	85 (38.8)
Clinical symptoms	
Fever	103 (47.0)
Urinary burning	38 (17.3)
Dysuria	18 (8.2)
Antibiotic therapy	
Yes	65 (29.7)
No	154 (70.3)

Bacteriological data:

Urine cytology revealed leukocyturia (> 10^4 WBC/mm^3) in 24.2% (53/219). Gram staining revealed the presence of bacteria in 17.8% (39/219) of cases. These were Gram-negative bacilli in 89.7% (35/39) and Gram-positive cocci in 10.3% (4/39) of cases. The bacterial culture positivity rate was 16.34% (36/219).

According to the Kass criteria, 28 voided urine samples met the criteria for UTI i. e. 12.8% (28/219). These infections were community-acquired in 25.0% (7/28), and hospital-acquired in 75.0% (21/28) of cases (Table 2). The infections also occurred more frequently in patients who have not been on antibiotic treatment but no significant statistical relationship (p=0.3).

The prevalence of UTI was significantly higher in the females (19.5%, 17/85) than the males (8.2%, 11/134) (OR 2.8, p=0.01) (Table 2). Urinary tract abnormalities were observed in 21.4% (6/28). The median age of patients presenting with a UTI was 96 months with range from 2 to 168 months.

The bacterial groups isolated were Enterobacteriaceae (89.3%, n=25) followed by enterococci (7.1%, n=2) and staphylococci (3.6%, n=1). *Escherichia coli* (60.7%, n=17) was the most frequent isolate, followed by *Klebsiella pneumoniae* (17.9%, n=5) and *Enterobacter* spp (7.1%, n=2). Among the Gram-positive cocci, *Enterococcus faecalis* (7.1%, n=2) and *Staphylococcus saprophyticus* (3.6%, n=1) were isolated (Table 3).

Table 2: Epidemiological and clinical characteristics of children with urinary tract infections

Variable	Number (%)	x^2	OR (95% CI)	*p* value
Kass criteria				
Yes	28 (12.8)			
No	191 (87.2)			
Origin				
Hospital (n=165)	21 (12.7)	0.002	1.0 (0.4-2.5)	1.00
Community (n=54)	7 (12.9)			
Sex				
Male (n=134)	11 (8.2)	5.47	2.8 (1.3 - 6.3)	0.01*
Female (n=85)	17 (19.5)			
Age group (years)				
0-2 (n=45)	7 (15.6)	1.9		0.39
2-5 (n=63)	5 (7.9)			
6-15 (n=111)	16 (14.4)			

x^2 = Chi square; OR = Odd ratio; CI = Confidence interval; * = statistically significant

Table 3: Bacterial isolates of urinary tract infections

Uropathogens	Number (%)
Enterobacteriaceae	**25 (89.3)**
Escherichia coli	17 (60.7)
Klebsiella pneumoniae	5 (17.9)
Enterobacter spp	2 (7.1)
Salmonella spp	1 (3.6)
Gram-positive cocci	**3 (10.7)**
Enterococcus faecalis	2 (7.1)
Staphylococcus saprophyticus	1 (3.6)
Total	**28 (100.0)**

Antibiotic sensitivity of isolated bacteria:

The rate of resistance of Enterobacteriaceae to beta-lactam antibiotics was over 50%. The rates for ampicillin and amoxicillin-clavulanic acid were 88.0% and 48% respectively. Susceptibility to 3rd generation cephalosporins was also affected, with resistance levels rising to 64.0% for ceftriaxone and cefotaxime respectively (Table 4).

Resistance to fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamycin and amikacin) were 52.0%, 52.0% and 32.0% respectively. Resistance of Enterobacteriaceae to imipenem (16.0%) and meropenem (12%) remained low.

Escherichia coli, the most frequent Enterobacteriaceae isolated, expressed high levels of resistance to amino-penicillins (93.8%), amoxicillin-clavulanic acid (56.3%) and cotrimoxazole (87.5%). Resistance to cefotaxime and ceftriaxone were 75.0% and 62.3% respectively (Table 5).

Thirty-two percent (8/25) of the Enterobacteriaceae were extended-spectrum beta-lactamase (ESBL) producers. These were *E. coli* (62.5%; n=5/8), *Klebsiella pneumoniae* (25.0%, n= 2/8), *Enterobacter* spp (12.5%, n=1/8).

Enterococcus faecalis strains were all sensitive to ampicillin, but 50.0% were resistant to norfloxacin, chloramphenicol and gentamicin (high level of resistance to aminoglycosides). Resistance to cyclins (minocycline, tetracycline) was 100.0%. *Staphylococcus saprophyticus* strain was resistant to penicillin G (100.0%), sulfamethoxazole-trimethoprim (100.0%) and cyclins (100.0%). All Gram-positive cocci were sensitive to vancomycin (Table 4).

Discussion:

Antibiotic resistance is a growing public health problem, which has an impact on the choice of first-line treatments for the most common infections, such as paediatric urinary tract infections (12). As a result, international organizations and associations such as the European Centre for Disease Prevention and Control (ECDC), the Infectious Diseases Society of America (IDSA) and the American Association of Pediatrics (AAP) consider it essential to disseminate local information on antimicrobial resistance in order to develop our own treatment guidelines (12,13).

The results of our study show that data were obtained from 219 patients, 75.4% of whom were hospitalised and 24.6% ambulatory. The most frequent symptoms for ECBU request in children was fever (47%), followed by urinary burning (17.3%) and dysuria (8.2%). Similar data had been reported by Cissé et al (14) who, in a study carried out in 2014 on 202 patients, reported the following as the main presenting symptom; fever in 100% of cases, digestive problems in 80.7% of patients and no urinary symptoms (7.05%).

Antibiotic therapy has been initiated in 29.7% of the patients in our study. The compliance and appropriateness of these antibiotic prescriptions had not been studied. Our rate is lower than that of Mabiala et al., (15) in the Congo, who in 2009 found a hospital prevalence of antibiotic prescribing in paediatrics of 61.5%, with beta-lactam antibiotics accounting for 79.0% of prescriptions (15).

Twenty-eight of 219 (12.8%) children in our study met the criteria for UTI, with

Table 4: Resistance rates of the bacterial isolates against the antibiotics tested

Antibiotic	**Bacteria isolates**		
	Enterobacteriaceae n=25 (%)	***Enterococcus faecalis* n=2 (%)**	***Staphylococcus saprophyticus* n=1 (%)**
Chloramphenicol	3 (12.0)	1 (50.0)	-
Minocycline	12 (48,0)	2 (100.0)	1 (100.0)
Ampicillin	22 (88.0)	0	-
Amoxicillin-clavulanic acid	12 (48.0)	-	-
Cefotaxime	16 (64.0)	-	-
Ceftriaxone	16 (64.0)	-	-
Imipenem	4 (16.0)	0	-
Norfloxacin		1 (50.0)	-
Ciprofloxacin	13 (52.0)	1 (50.0)	
Gentamicin	13 (52.0)	1 (50.0)	
Amikacin	8 (32.0)	-	0
Vancomycin	-	0	0
Cotrimoxazole	20 (80.0)	2 (100.0)	1 (100.0)
Penicillin G	-	-	1 (100.0)
Cefoxitin	-	-	0
Fusidic acid	-	-	1 (100.0)

Table 5: Resistance rates of *Escherichia coli* and *Klebsiella pneumoniae* isolates to the antibiotics tested

Antibiotic	***Escherichia coli* [n=16 (%)]**	***Klebsiella* pneumoniae [n=5 (%)]**
Chloramphenicol	1 (6.3)	1 (20.0)
Minocycline	9 (56.3)	3 (60.0)
Ampicillin	15 (93.8)	*RN
Amoxicillin-clavulanic acid	9 (56.3)	2 (40.0)
Cefotaxime	12 (75.0)	2 (40.0)
Ceftriaxone	10 (62.3)	3 (60.0)
Imipenem	3 (18.8)	0
Ciprofloxacin	8 (50.0)	4 (80.0)
Gentamicin	7 (43.8)	3 (60.0)
Amikacin	5 (31.2)	3 (60.0)
Cotrimoxazole	14 (87.5)	4 (80.0)

60.7% of them being females. The prevalence of UTI in the females (19.5%) was significantly higher than that in the males (8.2%) (OR 2.8, *p*=0.01). The prevalence of UTI was also higher among infants (1 month to 2 years) (15.6%) and children 6-15 years (14.4%) compared to children in the age group 2-5 years (7.9%) but the difference was not statistically significant (*p*=0.39).

Our findings are similar to those of Ouedraogo et al., (7) in Burkina Faso, who reported a frequency of paediatric UTI of 18.7%, with a predominance of females. Bouskraoui et al., in Morocco (16) reported a low frequency of paediatric UTIs of 1.33%, with a clear predominance of females at 65.7% (16). The female predominance is in line with data in the literature according to which 8% of females compared with 2% of males are likely to contract a UTI before the age of 7 years (17).

Furthermore, the higher susceptibility of females to UTIs could be explained by the relatively shorter length of the female urethra and the regular colonisation of the perineum by enteric organisms (18). In addition, UTIs occurred more frequently in hospitalised children (75%), which corroborates a previous study that reported UTIs accounting for 40% of all nosocomial infections (19).

The bacteriological profile of UTI in this study was dominated by Enterobacteriaceae in 89.3% of cases, with *E. coli* (60.7%) being the most frequent bacterium, followed by *Klebsiella pneumoniae* (17.9%). Several studies throughout the world confirm this trend. Bouskraoui (16) and Ferjani (20), from Morocco and Tunisia respectively, reported *Escherichia coli* in 72-80.4% of cases, followed by *Klebsiella* spp (9.8-14%) and *Proteus mirabilis*

(5.9%). Similarly in Saudi Arabia, Tahir et al., (21) reported *E. coli* to be the main uropathogen (75.7%), followed by *Klebsiella pneumoniae* (9.4%) and *Pseudomonas aeruginosa* (5.9%).

Enterococcus spp (3.5%) was the main Gram-positive cocci, which agrees with studies in the literature (22,23), which reported that *E. coli* accounts for 80-90% of urinary tract infections in children. In fact, majority of UTIs result from bacteria ascending from the periurethral zone, migrating retrograde via the urethra to reach the bladder and potentially the upper urinary tract.

With regard to the sensitivity of the bacteria to antibiotics, the Enterobacteriaceae were resistant to aminopenicillins (88.0%), penicillin+inhibitor combination (48.0%) and 3rd generation cephalosporins such as cefotaxime and ceftriaxone (64.0%), and 52.0% expressed resistance to quinolones. Similar data was reported by Boni (5) in Côte d'Ivoire, with high rates of resistance to the amoxicillin-clavulanic acid combination (51-85%) and to ciprofloxacin (64-75%).

Carbapenems remained the most active antibiotics against Enterobacteriaceae. *E. coli,* the main Enterobacteriaceae isolated, expressed high levels of resistance to aminopenicillins (93.8%), amoxicillin-clavulanic acid (56.3%) and cotrimoxazole (87.5%). Resistance of the isolates to cefotaxime, ceftriaxone and cefepime remained high.

Conclusion:

Paediatric UTIs are frequent and particularly affect infants and children at Bouaké University Hospital. Antibiotics such as ampicillin, amoxicillin, amoxicillin-clavulanic acid and ceftriaxone are no longer effective as first-line empirical treatment of paediatric UTIs in Bouaké. There is need to set up a multidisciplinary antibiotic stewardship team in Bouaké University Hospital to promote appropriate use of antimicrobials.

Contributions of authors:

GKJ and AC designed the study; OKH, AIA, VM, KMO, and TF carried out the surveys; GKJ, TJON, VM, and NM carried out the experimental work; GKJ, NM, and MP analysed the data and wrote the manuscript; AC, GKJ, TJON, NM and MP participated in the critical review of the manuscript. All authors approved the final manuscript submitted for publication.

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No conflict of interest is declared

References:

1. Mutombo, C. K., Mukandila, A. M., Mikenji, J. B., Katumbayi, G. N., and Kashi, F. M. Bacteriological profile of gravid urinary tract infection (Case of Bonzola Hospital in Mbujimayi, DR Congo). Rev Med Madag. 2015; 5 (3): 626-633.
2. Buettcher, M., Trueck, J., Niederer-Loher, A., et al. Swiss consensus recommendations on urinary tract infections in children. Eur J Pediatr. 2021;180(3):663-674. https://doi.org/10.1007/s00431-020-03714-4
3. Joan, L. R., Jane, C. F, Mia, E. L., and Robert, B. Canadian Paediatric Society. Diagnosis and management of urinary tract infections in infants and children. Paediatr Child Health. 2014;19 (6): 320-325. doi:10.1093/pch/19.6.315
4. Lavigne, J. P., Moing, V. L., and Sotto, A. Which antibiotics to use in routine practice for community-acquired urinary tract infections in France. Spectra Biologie. 2005; 24 (146}: 18-23.
5. Boni, C., Zaba, F., Meite, S., et al. Profil bactériologique des infections urinaires en milieu pédiatrique: cas du chu de Yopougon. J Sci Pharm Biol.2015; 16: 34-41.
6. Zahir, H., Draiss, G., Rada, N., et al. Microbial ecology and antibiotic susceptibility of bacteria isolated from urinary tract infections in children in Morocco. Rev Francoph Lab. 2019; 2019 (511): 65-70. https://doi.org/10.1016/S1773-035X(19)30229-1
7. Ouédraogo-Yugbaré, S., Kouéta, F., Dao, L., et al. Urinary tract infection in children: epidemiological and bacteriological aspects at the Centre Hospitalier Universitaire Pédiatrique Charles de Gaulle de Ouagadougou (Burkina Faso). Mali Med. 2012; 27 (4): 11-17.
8. Amorissani, M. F., M'bengue, A. K., Dainguy, E., et al. Neonatal urinary tract infections: clinical and bacteriological profiles. Int J Med Sci. 2006; 8 (1):45-49.
9. Sreenarasimhaiah, S., and Hellerstein, S. Urinary tract infections per se do not cause end-stage kidney disease. Pediatr Nephrol. 1998; 12(3): 210-213. https://doi.org/10.1007/s004670050439
10. Courcol, R., Marmonier, A., and Piemont, Y. Les difficultés d'interprétation de l'examen cyto-bactériologique des urines. Rev Francoph Lab. 2005; 2005 (370): 21-25. https://doi.org/10.1016/S0338-9898(05)80032-0
11. Cattoir, V., Denis, F., Martin, C., Ploy, M. C., and Poyart, C. Bactériologie médicale: Techniques usuelles. Elsevier Health Sciences; 2016: 599
12. Rosado, M. R., Molina, A. G., Velasco, A. L., et al. Urinary Tract Infection in Pediatrics: Study of Uropathogens and Their Resistance in a Madrid Hospital. Arch Esp Urol. 2022; 75 (9): 791-797. https://doi.org/10.56434/j.arch.esp.urol.20227509.115
13. Roberts, K. B. Subcommittee on Urinary Tract Infection SC on QI and M. Urinary Tract Infection: Clinical Practice Guideline for the Diagnosis and Management of the Initial UTI in Febrile Infants and Children 2 to 24 Months. Pediatr. 2011; 128 (3):595-610. https://doi.org/10.1542/peds.2011-1330
14. Cissé, L., Lagou, D., Ouattara, G. J., et al. Childhood urinary tract infection during a febrile attack at Port-Bouët General Hospital (Abidjan Ivory Coast). Rev Afr Malg. 2017; 5 (1): 105-109.
15. Babela, J. R. M., Ikobo, L. C. O., Cardorelle, A. M., and Moyen, G. Evaluation of initial antibiotic

therapy in paediatric settings at Brazzaville University Hospital (Congo). Trop Med Health. 2013; 23 (2): 189-192. https://doi.org/10.1684/mst.2013.0173

16. Bouskraoui, M., Ait Sab, I., Draiss, G., Bourrouss, M., and Sbihi, M. Epidemiology of urinary tract infection in children in Marrakech. Arch Pédiatr. 2010; 17: S177-S178. https://doi.org/10.1016/S0929-693X(10)70921-0
17. Millner, R., and Becknell, B. Urinary tract infections. Pediatr Clin North America. 2019; 66 (1): 1-13. https://doi.org/10.1016/j.pcl.2018.08.002
18. Leung, A. K., and Robson, W. L. Urinary tract infection in infancy and childhood. Adv Pediatr. 1991; 38: 257-285.
19. Kalsi, J., Arya, M., Wilson, P., and Mundy, A. Hospital-acquired urinary tract infection. Int J Clin Pract. 2003; 57 (5): 388-391.
20. Ferjani, A., Mkaddemi, H., Tilouche, S., et al. Epidemiological and laboratory characteristics of urinary tract infection. Arch Ped. 2011; 18(2): 230-234. https://doi.org/10.1016/j.arcped.2010.09.024
21. Tahir, H, Al Nafeesah A, Chishti S, Al Shaalan M, Al Fakeeh K. Community-acquired urinary tract infections in children: Resistance patterns of uropathogens in a tertiary care center in Saudi Arabia. Int J of Ped & Adolescent Med. 2019;6(2):51-4. https://doi.org/10.1016/j.ijpam.2019.02.010
22. Leung AKC, Wong AHC, Leung AAM, Hon KL. Urinary Tract Infection in Children. Rec Pat Inflamm Allerg Drug Discov. 2019; 13 (1): 2-18. https://doi.org/10.2174/1872213X13666181228154940
23. Clark CJ, Kennedy WA, Shortliffe LD. Urinary Tract Infection in Children: When to Worry. Urol Clin North Am. 2010; 37 (2): 229-241. https://doi.org/10.1016/j.ucl.2010.03.009

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Original Article **Open Access**

Prevalence, molecular characteristics and antibiotic susceptibility of clinical isolates of *Clostridioides difficile* in southeastern Nigeria

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Abstract:

Background: *Clostridioides difficile* is a major contributor to healthcare-associated infections, exhibiting global variations in its prevalence and resistance. There is an absence of data on its molecular characteristics and antibiotic susceptibility of *C. difficile* in southeastern Nigeria. This study aims to evaluate its prevalence, molecular features, and resistance patterns to enhance treatment and control strategies.
Methodology: Stool samples were collected from 450 hospitalized adult patients with persistent diarrhoea and prolonged antibiotic use from selected public and private healthcare facilities across the five States of south-eastern Nigeria. The total anaerobes were enumerated using standard bacteriological methods. *Clostridioides difficile* was cultured on *Clostridium difficile* differential agar with selective supplements, followed by biochemical testing including catalase, oxidase, and reverse CAMP tests. Genomic bacterial DNA was extracted, purified, and amplified by polymerase chain reaction (PCR) assay. Sequencing of the DNA amplicon was performed and results were blasted on the NCBI database. Virulence (*tcdA, tcdB, cdtA*, and *cdtB*) and resistance (*tetS, tetA,* and *ermB*) genes were detected by conventional PCR assay. Antibiotic susceptibility of each isolate was determined using the Kirby-Bauer disc diffusion method.
Results: The mean anaerobe counts ranged from 5.61±0.11 log10 CFU/g (Ebonyi) to 5.65±0.07 log10 CFU/g (Enugu). Antibiotic susceptibility test revealed sensitivity to gentamicin, meropenem, and amoxicillin-clavulanic acid, while resistance was observed to tetracycline and erythromycin. The multiple antibiotic resistance (MAR) index of isolates was 0.44. All isolates (100%) harbored *tcdB* gene while 69.56% harbored *tcdA* gene. Additionally, 4.34% of isolates contained *cdtA* and *cdtB* genes.
Conclusions: This study highlights significant antibiotic resistance and presence of virulence genes in *C. difficile* isolates in southeastern Nigeria, emphasizing the need for monitoring and tailored treatment strategies.

Keywords: Prevalence; Molecular; Antibiotic susceptibility; *Clostridioides difficile*; southeast Nigeria

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Prévalence, caractéristiques moléculaires et sensibilité aux antibiotiques des isolats cliniques de *Clostridioides difficile* dans le sud-est du Nigéria

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Résumé:

Contexte: *Clostridioides difficile* est un contributeur majeur aux infections associées aux soins de santé, présentant des variations mondiales dans sa prévalence et sa résistance. Il n'existe pas de données sur ses caractéristiques moléculaires et la sensibilité aux antibiotiques de *C. difficile* dans le sud-est du Nigéria. Cette étude vise à évaluer sa prévalence, ses caractéristiques moléculaires et ses profils de résistance afin d'améliorer les stratégies de traitement et de contrôle.
Méthodologie: Des échantillons de selles ont été prélevés auprès de 450 patients adultes hospitalisés souffrant de diarrhée persistante et d'une utilisation prolongée d'antibiotiques dans des établissements de santé publics et privés sélectionnés dans les cinq États du sud-est du Nigéria. Le nombre total d'anaérobies a été dénombré à l'aide de méthodes bactériologiques standard. *Clostridioides difficile* a été cultivé sur gélose différentielle *Clostridium difficile* avec des suppléments sélectifs, suivi de tests biochimiques comprenant des tests de catalase, d'oxydase et de CAMP inverse. L'ADN bactérien génomique a été extrait, purifié et amplifié par un test de réaction en chaîne par polymérase (PCR). Le séquençage de l'amplicon d'ADN a été effectué et les résultats ont été enregistrés dans la base de données NCBI. Les gènes de virulence (*tcdA*, *tcdB*, *cdtA* et *cdtB*) et de résistance (*tetS*, *tetA* et *ermB*) ont été détectés par un test PCR conventionnel. La sensibilité aux antibiotiques de chaque isolat a été déterminée à l'aide de la méthode de diffusion sur disque de Kirby-Bauer.
Résultats: Le nombre moyen d'anaérobies variait de 5,61±0,11 log10 UFC/g (Ebonyi) à 5,65±0,07 log10 UFC/g (Enugu). Le test de sensibilité aux antibiotiques a révélé une sensibilité à la gentamicine, au méropénème et à l'amoxicilline-acide clavulanique, tandis qu'une résistance a été observée à la tétracycline et à l'érythromycine. L'indice de résistance multiple aux antibiotiques (MAR) des isolats était de 0,44. Tous les isolats (100 %) abritaient le gène *tcdB* tandis que 69,56% abritaient le gène *tcdA*. De plus, 4,34% des isolats contenaient les gènes *cdtA* et *cdtB*.
Conclusions: Cette étude met en évidence une résistance importante aux antibiotiques et la présence de gènes de virulence dans les isolats de *C. difficile* dans le sud-est du Nigéria, soulignant la nécessité d'une surveillance et de stratégies de traitement adaptées.

Mots clés: Prévalence; Moléculaire; Sensibilité aux antibiotiques; *Clostridioides difficile*; sud-est du Nigéria

Introduction:

Standard healthcare facilities are essential for survival in a world dominated by superbugs, which have also been implicated in infections and the re-occurrence of infection even after the treatment regimen. Pathogenic organisms have recorded unparalleled success in their ability to cause diseases as well as induce therapeutic failure through drug resistance (1). *Clostridioides difficile* has been reported as the leading cause of healthcare-associated diarrhea (2). Antibiotic exposure, which reduces the population of non-pathogenic anaerobes that normally inhabit the gut, has been pointed out as the most important risk factor for *C. difficile* infection (CDI) (2). Thus, CDI occurs when there is a shift in the microbial flora of the colon, allowing toxin-producing strains of Gram-positive, spore-forming, anaerobic bacillus to proliferate (2). Other events implicated in gut microbiota disruption include proton pump inhibitors (PPI) use and inflammatory bowel diseases (3,4). Researchers have attributed the pathogenicity of *C. difficile* to the two major virulence factors produced by toxigenic *C. difficile* strains; enterotoxin A (TcdA) and cytotoxin B (TcdB) (3,4).

On September 16, 2013, the Centre for Disease Control (CDC) declared *C. difficile* as a threat with 'urgent' concern to public health in the United States due to the identification of antimicrobial resistance among human and animal-derived *C. difficile* isolates to drugs commonly used in humans and also to their increased rates of associated patient mortality since the early 2000s (5). In all the studies, some strains of *C. difficile* appear to be species-specific, while other strains were isolated from multiple species, including man. The organism was initially described as part of the normal flora in stool samples from healthy infants in 1935 (6) as well as its detection in significant numbers in healthy asymptomatic children (7). It was afterward identified as a pathogen associated with several diseases, and even now, in hospitals and long-term care facilities, it has become the most common cause of diarrhea, causing the loss of billions of dollars in health care costs (8).

The symptoms of CDI usually appear mild in some cases, and in others, they appear to be life-threatening or fatal (9). Diarrhea associated with *C. difficile* is accompanied by the passage of occult blood or mucus in the stool, but hematochezia and melena are other rare symptoms (4,10). Certain other symptoms are found in less than 50% of infected patients, including abdominal discomfort, fever, and peripheral leukocytosis (11). Disease caused by *C. difficile* is linked most commonly to nosocomial infections in humans, especially after empiric antibiotic treatment. Humans develop a spectrum of diseases when infected with some strains of *C. difficile*. It has been shown that not all people who are on prolonged antibiotic medication and above the age of 60 years are susceptible to *C. difficile* (12).

The advent of some virulent new strains of *C. difficile* has triggered an increase in CDI rates even in younger populations originally thought to be at low risk of developing CDI (13). To date, little is known about CDI in southeastern Nigeria as inadequate attention

has been given to the understanding of the molecular epidemiology of CDI in Nigeria, a high-burden country. Thus, this research was aimed at determining the prevalence, molecular characteristics, pathogenicity, and antimicrobial susceptibility of *C. difficile* isolated from clinical specimens in the southeastern States of Nigeria.

Materials and method:

Study area:

This study was conducted at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra State, and hospitals selected from the other four neighboring States of southeastern Nigeria.

Ethics approval and consent to participate:

The study was conducted in line with the principles of the Declaration of Helsinki. Ethical approval for this research was obtained from Ethical Committee, Chuckwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku, Awka, Anambra State, Nigeria. The ethics approval number is COOUTH/CMAC/ETH. C/VOL.1/0035 and the approval was granted on 01/08/2018. The committee reviewed and approved all aspects of the study, including the study design, recruitment methods, and data collection procedures, ensuring that they were in compliance with ethical standards and guidelines.

The consent to participate was obtained from all individuals who participated in the study. Participants were given detailed information regarding the research objectives, procedures, potential risks, and benefits. They were informed that participation was voluntary, that they could withdraw from the research at any given time without any punishment, and their data would be given full confidentiality.

Study participants and sample size:

Participants included a total of 450 hospitalized patients who exhibited symptoms such as watery stools (unformed stools in 24 hours), abdominal pain, and fever. Stool samples were collected from symptomatic patients hospitalized in various units, including the intensive care unit (ICU), medical, surgical and gastroenterology wards. These units were selected due to the high prevalence of CDI, particularly among patients receiving prolonged antibiotic therapy or those with underlying comorbidities. Additionally, samples were obtained from patients at the infectious disease unit who were undergoing evaluation for suspected CDI. All participants had diarrhea (unformed stools in 24 hours) and were tested for *C. difficile* based on clinical suspicion.

The sample size was determined using the sample size formula; $N=Z^2 p (1-p)/D^2$ (14) where, N is the sample size, Z is the statistic for level of confidence (1.96 for 95% confidence level), p is the prevalence rate of 45% (0.45) based on a previous study and D is the desired level of precision (0.05). The sample size of 384 obtained from the formula was increased to 450 to account for environmental differences and sampling challenges.

Sample collection:

Stool samples were collected in red cap universal bottles from the Chukwuemeka Odumegwu Ojukwu University Teaching hospital, Awka in Anambra, Federal Teaching hospital Abakaliki in Ebonyi, Abia State University Teaching hospital in Abia, Imo State University Teaching hospital in Imo and Enugu State Teaching hospital in Enugu. Following initial processing, samples were properly stored and preserved on ice packs to maintain sample integrity until further analysis.

Isolation and identification of *Clostridium difficile*:

For the isolation of *C. difficile*, all samples were pre-treated (heat shocked and alcohol shocked) to reduce competing flora and allow the growth of spore-forming bacilli. Standard microbiological method was employed using *C. difficile* differential agar CM 0601 (Oxoid, UK) supplemented with *C. difficile* selective supplement SR0096 (Oxoid, UK) (15).

Stock solutions were prepared for the samples by weighing 20g of the stool samples into 180ml of sterile saline water in a conical flask. A ten-fold serial dilution from the stock solution was made after mixing. From the fifth tube, 2ml of the suspension was added to a well-labelled tube containing 2ml of absolute ethanol (alcohol/ethanol shock method). The mixture was rocked gently to allow for homogenization and left to stand for an hour before plating of 100 µL was carried out.

Heat treatment, also known as heat shocking, was employed by heating the tubes containing a mixture of samples to boil for 5 minutes. The tubes were then allowed to cool before plating was carried out on the media. Since *C. difficile* is a strict anaerobe, its growth requires an oxygen-free environment. All inoculated plates were incubated in anaerobic condition using anaerobic jar with gas-generating sachets to create a completely oxygen-free atmosphere at 37°C for 48–72 hours to ensure optimal recovery of *C. difficile*.

The formula employed for the dilution factor is given by the equation; final volume/ aliquot volume, where final volume is aliquot volume (sample volume) plus diluent volume. Enumeration of the bacterial isolates was carried out using the formula described by Willey et al., (16); CFU/g = (number of colonies x dilution factor)/volume of inoculum. The cultu-

ral, morphological, microscopic and biochemical properties of the isolates were evaluated after Gram staining, and spore staining was done to select the positive isolates for both stains. Biochemical tests such as catalase, oxidase, indole, urease and sugar fermentation tests were carried out to identify *Clostridium* species.

Antimicrobial susceptibility test:

Antimicrobial susceptibility test (AST) was performed on identified *C. difficile* by the disc diffusion method. The antibiotic discs (Oxoid, UK) used include meropenem (10µg), erythromycin (15µg), metronidazole (50µg), amoxicillin-clavulanic acid (20/10µg), clindamycin (20µg), gentamicin (10µg), ciprofloxacin (5µg), vancomycin (30µg), and tetracycline (30µg).

Briefly, 18-24 hours bacterial culture was cultured on Mueller-Hinton agar (MHA). The inoculum was standardized to 0.5 McFarland standard (~1.5×10^8 cells/ml) was streaked onto the MHA plate with a sterile loop before the antibiotic discs were aseptically placed on the plates using a sterile forcep. The plates were incubated under strict anaerobic conditions (90% N_2, 5% H_2, 5% CO_2) at 35–37°C for 24–72 hours to ensure proper growth for AST. The diameter of zone of inhibition was measured and the result was interpreted using the Clinical and Laboratory Standards Institute (CLSI) breakpoints (17).

Determination of multiple antibiotic resistance (MAR) index:

The MAR index for an isolate was determined by the formula described by Chitanand et al., (18); y/nx, where y is the number of antibiotics the isolate is resistance to, n is the number of isolates tested, and x is the total number of antibiotics tested.

DNA extraction:

Bacterial DNA was extracted by the boiling method (19). A tube containing 2ml of a 24-hour broth culture of *C. difficile* was centrifuged at high-speed for 5 minutes. After the supernatant was removed, 200µL of sterile distilled water (SDW) was added to the pellets, and the mixture was vortexed for 1 minute. After the mixture was heated to 100°C for 15 minutes, it was centrifuged at high-speed for 2 minutes. The supernatant from this was considered pure DNA, and 10µL of it was used for amplification of the 16S rDNA as well as the virulence and resistance genes by PCR.

DNA sequencing:

The amplified bacterial 16S rDNA fragments were sequenced using the Genetic Analyzer (Model 3130xl) from Applied Biosystems (20). The sequencing reactions were performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit, following the manufacturer's recommended protocol to ensure optimal sequencing conditions (21). All procedures were conducted according to standard laboratory protocols to maintain data accuracy and reliability and the sequences were blasted on the website of National Centre for Biotechnology Information (21).

Phylogenetic analysis:

Phylogenetic analysis was performed by using the amplified DNA to determine the evolutionary relationships among the isolates. DNA sequences were initially aligned using both ClustalW and MUSCLE to ensure precision and accurate comparisons (22). The aligned sequences were then used to construct phylogenetic trees, which were visualized and edited using MEGA 10 software (23). This software also facilitated calculation of bootstrap values, providing an assessment of the reliability and robustness of the phylogenetic trees. The analysis included both Neighbor-Joining and Maximum Likelihood methods to confirm the consistency of the results (24).

PCR amplification for detection of virulence and resistance genes:

Using standardized primer sets, virulence genes (*tcdA*, *tcdB*, *cdtA*, and *cdtB*) were detected in duplicate to ensure that the results were accurate using the primers listed in Table 1. The analysis utilized the extracted bacterial genomic DNA as template. The PCR assay was performed in tubes containing 50 mM KCl_2, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM dNTPs, and 20 pmol of each primer along with 2U Taq DNA polymerase in a total volume of 50µL, using 4µL of the extracted DNA as template (25).

To ensure the primers specifically bind to the DNA template, the thermocycler was optimized to ascertain the correct annealing temperature. The PCR protocol consisted of an initial denaturation step at 94°C for 5 minutes, then 34 cycles involving 30 seconds of denaturation at 94°C, 1 minute of annealing at 55°C, and 1 minute of extension at 72°C. At completion of the amplification cycles, a final extension at 72°C for 5 minutes was performed. Sterile water served as the negative control to confirm that no contamination was present (3).

With conventional PCR assay, the genes responsible for erythromycin resistance (*ermB*) and tetracycline resistance (*tetA* and *tetS*) were detected using primers as shown in Table 2. The extracted bacterial genomic DNA was used as template for the analysis. The PCR assay was performed in tubes containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 200µM dNTPs, and 2U Taq DNA polymerase per 50µL reaction, using 4µL of extracted DNA as the template and including 20 pmol

of each primer (26).

The thermocycler was set to optimal annealing temperature that guaranteed specific binding of the primer set to the DNA template. The PCR condition included 5-minute pre-denaturation phase at 94°C, followed by 34 cycles, consisting of 30 seconds at 94°C (de-naturation), 45 seconds at 55°C (annealing), and 1 minute at 72°C (extension). After the amplification cycles, a final extension step at 72°C for 5 minutes was performed. Sterile water served as a negative control to eliminate the possibility of contamination.

Agarose gel electrophoresis:

Agarose gel electrophoresis was used to examine the PCR amplicons derived from primer sets targeting the virulence genes (27), as well those targeting the resistance (*ermB*, *tetA*, and *tetS*).

Statistical analysis:

Data were analyzed with infographics using Microsoft Excel (Version 2016, 2015, Microsoft Corporation, USA) (28). Comparisons of multiple means was done by analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 21.0 software. Phylogenetic tree analysis of species relatedness was done using Mega 10 software (Version 10, 2016, MEGA Limited, Japan)

Results:

The results of anaerobic stool sample counts from southeastern Nigeria are shown in Table 3. The total anaerobe counts (log_{10} CFU/g) in different States showed that stool samples from patients in Anambra, Ebonyi, Enugu and Imo had mean counts of 5.63±0.09 log_{10}CFU/g, 5.61±0.11 log_{10}CFU/g, 5.65±0.07 log_{10}CFU/g and 5.36±0.19 log_{10}CFU/g respectively. The identity and phylogenetic relatedness of anaerobic bacterial isolates obtained from stool samples in southeastern Nigeria are shown in Table 4.

The prevalence of anaerobes from stool samples using standard culture method revealed that all stool samples except from Imo State (46.0%) had anaerobic counts equal to or greater than 50.0% following a

Table 1: Primer sets for PCR amplification of the virulence genes

Genes	Primer	Sequence (5’-3’)	Concentration (µM)	Product size (bp)
tcdA	tcdA-F tcdA-R	GTATGGATAGGTGGAGAAGTCAGTG CGGTCTAGTCCAATAGAGCTAGGTC	0.025	632
tcdB	tcdB-F tcdB-R	GAAGATTTAGGAAATGAAGAAGGTGA AACCACTATATTCAACTGCTTGTCC	0.01	441
cdtA	cdtA-F cdtA-R	ATGCACAAGACTTACAAAGCTATAGTG CGAGAATTTGCTTCTATTTGATAATC	0.2	260
cdtB	cdtB-F cdtB-R	ATTGGCAATAATCTATCTCCTGGA CCAAAATTTCCACTTACTTGTGTTG	0.5	179

Table 2: Primer sets for PCR amplification of the resistance genes

Genes	Primer	Sequence (5’-3’)	Concentration (µM)	Product size (bp)
erm(B)	ermB-F ermB-R	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	0.1	639
tet(S)	tetS-F tetS-R	ATCAAGATATTAAGGAC TTCTCTATGTGGTAATC	0.1	573
tet(A)	tet(A)-F tet(A)-R	TTGGCATTCTGCATTCACTC GTATAGCTTGCCGGAAGTCG	0.1	494

cultural phenotypic standard (Table 5). In comparison, a total of 54.0% was obtained in the entire study.

The prevalence of *C. difficile* from stool samples revealed a total study prevalence of 3.1%. Stool samples from Ebonyi and Enugu States had a prevalence of 2.2% apiece. In comparison, Imo and Abia States had a 3.3% prevalence of *C. difficile* from stool samples, with Anambra State having the highest prevalence of 4.4%. The rate of *C. difficile* recovered by heat treatment method (1.1%) shown in Table 5 was lower than the rate recovered by alcohol treatment (3.1%).

Table 3: Total anaerobic bacterial count of stool samples obtained from patients in different states

Location	Number of Samples	Anaerobe count (Log_{10}CFU/g)	*p*-value(s)
Anambra	90	5.63 ± 0.09^{a}	0.168
Ebonyi	90	5.62 ± 0.11^{ab}	0.168
Enugu	90	5.65 ± 0.07^{a}	0.168
Imo	90	5.37 ± 0.19^{b}	0.066
Abia	90	5.45 ± 0.09^{b}	0.066

Table 4: Identity of anaerobic *Clostridioides difficile* isolates from stool samples from southeastern Nigeria

Sample code	Source	Identity	Query Cover (%)	Homology (%)	Accession number
anStool12	Stool	*Clostridioides difficile*	100	100.00	MH888201.1
ebstool05	Stool	*Clostridioides difficile*	98	91.4	CP019858.1
Imstool15	Stool	*Clostridioides difficile*	99	99.8	CP019860.1
anStool21	Stool	*Clostridioides difficile*	99	96.75	CP025047.1
anStool22	Stool	*Clostridioides difficile*	97	90.35	CP020424.2
abstool12	Stool	*Clostridioides difficile*	100	100.00	MH888202.1
abstool17	Stool	*Clostridioides difficile*	99	99.8	CP019860.1
imstool83	Stool	*Clostridioides difficile*	91	94.6	CP028361.1
imstool49	Stool	*Clostridioides difficile*	91	94.6	CP028361.1
ebstool23	Stool	*Clostridioides difficile*	91	94.6	CP028361.1
ebstool33	Stool	*Clostridioides difficile*	99	99.8	CP019860.1
ebstool1	Stool	*Clostridioides difficile*	99	96.75	CP025047.1
abstool12	Stool	*Clostridioides difficile*	97	90.35	CP020424.2
anstool70	Stool	*Clostridioides difficile*	100	100.00	MH888202.1

Table 5: Prevalence of anaerobes, *Clostridioides difficile* and percentage recovery of *Clostridioides difficile* from stool samples

Prevalence of anaerobes from stool samples using standard cultural methods								
Sample	Sample Number	Anambra	Ebonyi	Enugu	Imo	Abia	Total Sample	Study Total (%)
	90	55 (61)	45 (50)	50 (55)	41 (46)	50 (55)	450	241 (54)
Prevalence of *Clostridioides difficile* from stool samples in the study								
Stool sample	90	4 (44.4)	2 (22.2)	2 (22.2)	3 (33.3)	3 (33.3)	450	14 (3.1)
Percentage recovery of *Clostridioides difficile* from stool samples using cultural methods								
Sample	Number	Heat Treatment (%)		Alcohol Treatment (%)			Study Total (%)	
Stool sample	450	5 (1.1)		14 (3.1)			14 (3.1)	

In Table 6a and 6b, the antimicrobial susceptibility test of *C. difficile* isolates to the common antibiotics tested showed sensitivity to carbapenems (71.4%), aminoglycosides (78.5%) and β-lactam/combination agents (57.1%). Notable resistance was observed for tetracycline (57.1%), macrolides (57.1%) and fluoroquinolones (71.4%).

The multiple antibiotic resistance index of *C. difficile* isolates from the clinical samples showed that isolates with a MAR index of 0.44 had the highest resistance phenotypes. In contrast, isolates with a MAR index of 0.33 had the next highest resistance phenotypes (Table 7).

Table 6a: Antibiotic sensitivity profile of *Clostridioides difficile* to common antibiotics

Code	Samples	MEM	ERY	MET	AMC	CL	CN	CIP	VA	TET
anStool12	Stool	S	R	S	R	S	S	S	R	R
ebStool05	Stool	S	S	S	R	S	S	S	R	R
imStool15	Stool	S	R	R	S	R	S	R	S	R
anStool21	Stool	S	S	S	S	S	S	S	S	R
anStool22	Stool	S	R	S	S	S	S	R	S	R
abStool12	Stool	R	S	R	S	R	S	S	S	R
abStool77	Stool	S	R	R	S	S	S	R	S	R
imStool83	Stool	S	R	S	S	R	S	R	S	S
imstool49	Stool	S	R	R	S	R	S	R	S	R
ebstool23	Stool	R	R	R	S	S	S	S	S	R
enstool33	Stool	S	R	S	S	S	S	S	S	R
enstool1	Stool	S	R	S	S	R	S	R	S	R
abStool14	Stool	S	S	S	R	S	S	S	R	S
anstool70	Stool	S	S	S	S	S	S	R	S	S

Meropenem (MEM 10µg), Erythromycin (ERY 15µg), Metronidazole (MET 50µg), Amoxicillin/clavulanic acid (AMC 20/10µg), Clindamycin (CL 20µg), Gentamicin (CN 10µg), Ciprofloxacin (CIP 5µg), Vancomycin (VA 30µg), Tetracycline (TET 30µg), S = Sensitive, R = Resistance

Table 6b: Percentage of antibiotic resistance and susceptibility of *Clostridioides difficile* to common antibiotics

Antibiotic class	Antibiotic	Percentage susceptibility/resistance (n=14)	
		Sensitive (%)	Resistant (%)
Carbapenems	MEM	10 (71.4)	4 (28.5)
Macrolides	ERY	6 (42.8)	8 (57.1)
Nitroimidazole	MET	7 (50.0)	7 (50.0)
β-lactam/combination agents	AMC	8 (57.1)	6 (42.8)
Lincosamide	CL	6 (42.8)	8 (57.1)
Aminoglycosides	CN	11 (78.5)	3 (21.4)
Fluoroquinolone	CIP	4 (28.5)	10 (71.4)
Glycopeptides	VA	7 (50.0)	7 (50.0)
Tetracyclines	TET	6 (42.8)	8 (57.1)

Meropenem (10 µg), Erythromycin (15 µg), Metronidazole (50 µg), Amoxicillin/clavulanic acid (20/10 µg), Clindamycin (20 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Tetracycline (30 µg).

Table 7: Multiple antibiotic resistance distribution of *C. difficile* isolates from clinical sources

Isolate code	No of antibacterial classes	No of antibiotics	Resistance phenotypes	Number (%)	MAR Index
anBE7, ebstool05	9	9	AMCR, VAR, TETR	2 (8.7)	0.33
enLV4, ebstool23	9	9	MEMR, ERYR, METR, TETR	2 (8.7)	0.44
ebLV5	9	9	ERYR, CNR, TETR	1 (4.4)	0.33
anStool12	9	9	ERYR, AMCR, VAR, TETR	1 (4.4)	0.44
imstool15, imstool49	9	9	ERYR, METR, CLR, CIPR, TETR,	2 (8.7)	0.55
anstool21	9	9	TETR	1 (4.4)	0.11
anstool222	9	9	ERYR, CIPR, TETR	1 (4.4)	0.33
abstool12	9	9	MEMR, ERYR, METR, CLR, TETR	1 (4.4)	0.55
enstool33	9	9	ERYR, TETR	1 (4.4)	0.22
enstool1	9	9	ERYR, CLR, CIPR, TETR	1 (4.4)	0.44
abstool14	9	9	AMCR, VAR	1 (4.4)	0.22
anstool70	9	9	CIPR	1 (4.4)	0.11

Meropenem (MEM 10 µg), Erythromycin (ERY 15 µg), Metronidazole (MET 50 µg), Amoxicillin/clavulanic acid (AMC 20/10 µg), Clindamycin (CL 20 µg), Gentamicin (CN 10 µg), Ciprofloxacin (CIP 5 µg), Vancomycin (VA 30 µg), Tetracycline (TET 30 µg)

All the *C. difficile* isolates (100.0%) possessed *tcdB* genes, and 10 of 14 (71.4%) isolates possessed *tcdA* genes. Only one of the isolated *C. difficile* strains possessed *cdtA* and *cdtB* genes, thus giving a percentage of 7.1% apiece for the genes. The prevalence of virulence genes in Table 8 revealed that 3 isolates possessed (one of the four) virulence genes analyzed in the study, while 11 isolates possessed two of the four virulence genes.

Table 8: Prevalence of virulence genes in isolates of *C. difficile* obtained from stool samples

Lanes	Code	Samples	*tcdA*	*tcdB*	*cdtA*	*cdtB*	Total (%)
1	anStool12	Stool	+	+	-	-	2 (50.0)
2	ebstool05	Stool	+	+	-	-	2 (50.0)
3	Imstool15	Stool	+	+	-	-	2 (50.0)
4	anstool21	Stool	-	+	-	-	1 (25.0)
5	anstool22	Stool	+	+	+	+	4 (100.0)
6	abstool12	Stool	+	+	-	-	2 (50.0)
7	abstool77	Stool	+	+	-	-	2 (50.0)
8	imstool83	Stool	-	+	-	-	1 (25.0)
9	imstool49	Stool	+	+	-	-	2 (50.0)
10	ebstool23	Stool	-	+	-	-	1 (50.0)
11	enstool33	Stool	+	+	-	-	2 (50.0)
12	enstool11	Stool	-	+	-	-	1 (25.0)
13	abstool12	Stool	+	+	-	-	2 (50.0)
14	anstool70	Stool	+	+	-	-	2 (50.0)
	Total Study (%)		**10 (71.4)**	**14 (100.0)**	**1 (7.1)**	**1 (7.1)**	

The frequency and distribution of resistance genes detected in *C. difficile* obtained from stool samples are as shown in Table 9. Erythromycin resistance gene (*ermB*) was detected by PCR from *C. difficile* isolates in 64.2% of the samples while tetracycline resistance genes (*tetS* and *tetA*) were detected in 78.5% of the samples. Nine isolates possessed all resistance genes analyzed in the study.

Agarose gel electrophoresis of the amplified 16S rDNA of *C. difficile* isolated from the stool samples is shown in Fig 1 while the phylogenetic relatedness of representative *C. difficile* isolates with other anaerobic *Clostridium* species is shown in Fig 2.

Table 9: Prevalence of resistant genes to erythromycin and tetracycline found in isolates of *C. difficile* obtained from stool samples

Lanes	Code	Samples	*TetA*	*tetS*	*ErmB*	Total (%)
1	anStool12	Stool	+	+	+	3 (100.0)
2	ebstool05	Stool	+	+	-	2 (66.7)
3	Imstool15	Stool	+	+	+	3 (100.0)
4	anstool21	Stool	+	+	-	2 (66.7)
5	anstool22	Stool	+	+	+	3 (100.0)
6	abstool12	Stool	+	+	+	3 (100.0)
7	abstool77	Stool	+	+	+	3 (100.0)
8	imstool83	Stool	-	-	+	1 (33.3)
9	imstool49	Stool	+	+	+	3 (100.0)
10	ebstool23	Stool	+	+	+	3 (100.0)
11	enstool33	Stool	+	+	+	3 (100.0)
12	enstool1	Stool	+	+	+	3 (100.0)
13	abstool12	Stool	-	-	-	0
14	anstool70	Stool	-	-	-	0
	Total study (%)		**11 (78.5)**	**11 (78.5)**	**9 (64.2)**	

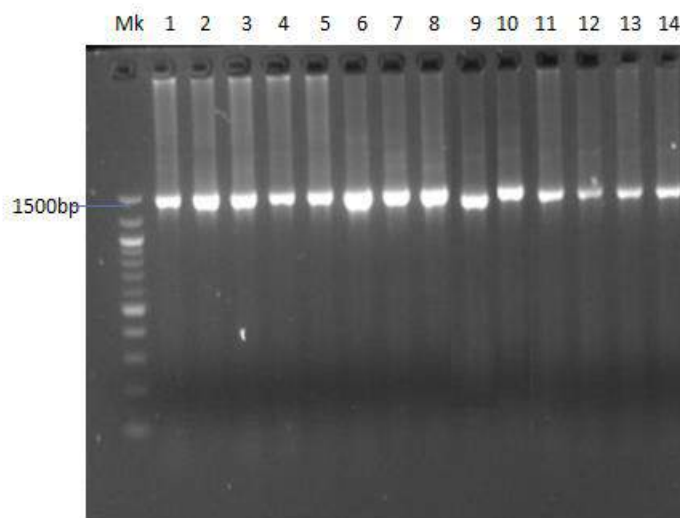

Fig 1: Agarose gel electrophoresis of amplified 16S rDNA of *Clostridioides difficile* isolated from stool samples

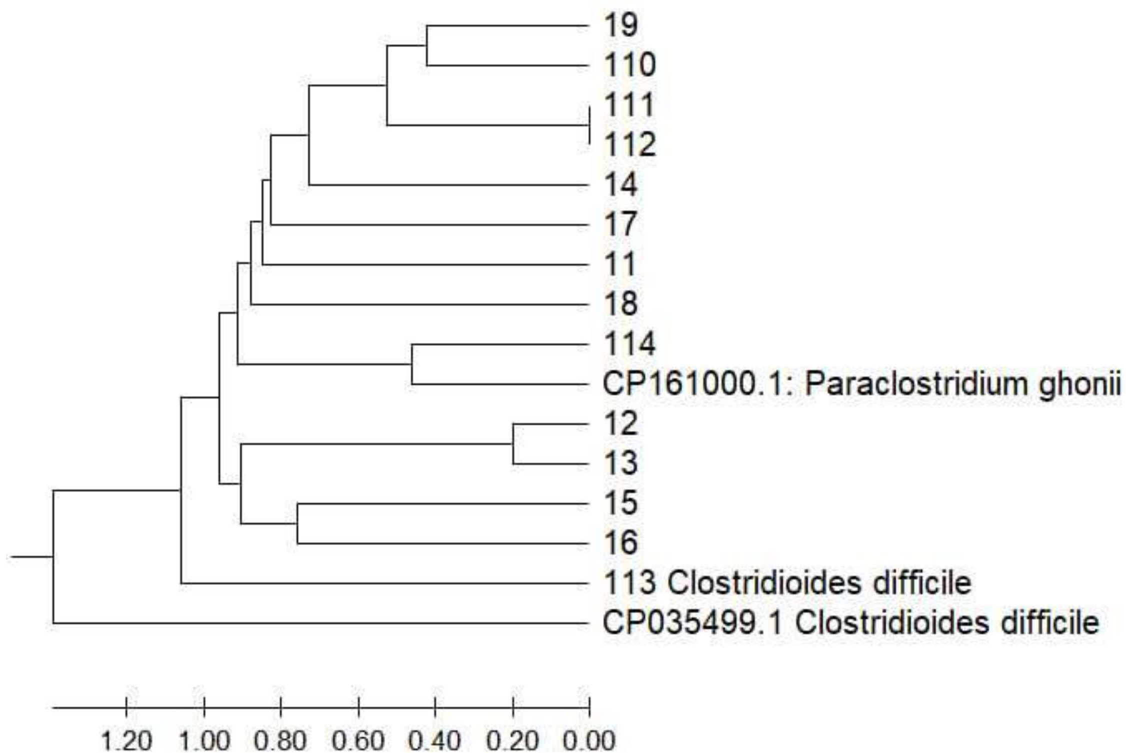

Fig 2: Phylogenetic relatedness amongst anaerobes isolates from clinical samples

Discussion:

A large portion of bacteria (anaerobes) in the stool are commensals, which often do not harm humans unless there is an underlying risk factor or the case of a compromised immune system. The bacterium of interest in this study (*C. difficile*) is regarded as both a commensal organism and a pathogen to domestic animals (29). Most of these anaerobes in the gut that have been isolated from the feces are not competitive, and their population can only explode, or they can thrive better in the gut after an array of antibiotic treatments which could destroy much of the intestinal normal flora (29). The statistical comparison of the total anaerobic count in stool samples showed no significant difference ($p>0.05$) between samples obtained from Ebonyi and other southeastern States of Nigeria in the study. However, there was a significant difference between samples obtained from Anambra and those from Imo and Abia States.

The bacterial identity, query cover, and percentage homology were written against the confirmed blasted identity of the isolates, including accession numbers obtained from the National Centre for Biotechnology Information (NCBI) website. The 3.1% prevalence of *C. difficile* obtained in this study is less than the rates reported by several studies in literature. This implies that the probability of infection due to *C. difficile* would be present. However, the presence of the pathogen (even in its low prevalence) in this study is an indication that there is a likely chance of a CDI outbreak if proper measures are not in place to curtail the infection.

Following reports of Warriner et al., (30), *C. difficile* was previously thought to be only clinically associated. However, with advanced scientific methods, CDI is now thought to be a community-acquired infection. The prolonged survival of spores of *C. difficile* in the environment might also increase the possibility of animal and food contamination which is a potential source of infection (31). In Europe, a higher prevalence had also been reported than the one obtained in this study, howbeit for hospitalized patients with even higher reports of toxigenic strains of *C. difficile* recovered from stool samples of patients (32). Nevertheless, findings in our study corroborated the report of others (33,34), who opined that carriage rates of *C. difficile* in healthy adults varied from 0% to 3% in Europe and up to 15% in Japan.

Following cultural phenotypic methods, the recovery rate of *C. difficile* from stool samples revealed that while both treatment methods (heat and alcohol treatments) yielded positive *C. difficile* growth, the alcohol shock method was better at recovering the bacterial isolates from clinical samples. The findings in this study about how the spores of the bacterium were recovered from both treatment processes agreed with the findings of Lawley et al., (35). They reported that spores are diffi-

cult to eradicate due to their ability to resist radiation, heat and chemicals (such as alcohols), which are usually employed as disinfectants in both hospital and community settings. In this study, alcohol shock samples had a better recovery rate of 3.1% than heat shock samples rate of 1.1%.

The susceptibility to carbapenems and aminoglycosides by most isolates of *C. difficile* in this study is at variance with what was observed in the study by Jobstl et al., (36), which separately reported some levels of resistance to the antibiotics by this bacterium. It is noteworthy that some of these antibiotics are commonly used for the treatment of diarrhea illnesses in humans. *Invitro* resistance of *C. difficile* to several classes of antimicrobial agents has been recognized and reported (37), thus, there is the possibility of a high risk for CDI to occur in healthcare settings and the community at large. This finding corroborated the report of Freeman et al., (38), who asserted that the resistance of *C. difficile* to several antibacterial agents may not be essential for CDI because the organism is relatively susceptible to high-ceiling antimicrobial agents.

It has been reported that the major pathogenic mechanism of *C. difficile* is production of cytotoxin B and enterotoxin A, which are respectively encoded by *tcdB* and *tcdA* genes, co-located in a 19.6 kb region of the chromosome named pathogenicity loci (Paloc) along with other regulatory genes (3,4). Toxin A causes diarrhea and Toxin B is cytotoxic to the colonic cells. Virtually all studies where *C. difficile* have been successfully recovered have screened for the ability of the isolates to have had at least one of the three toxins or genes (*tcdA, tcdB, cdtA/B*) needed to fulfill the criteria for virulence/toxigenicity (39). The findings in this study (71.4% and 100.0% for *tcdA* and *tcdB* genes respectively) agree with the study of Shokoohizadeh et al., (40), who opined that the genes for enterotoxin production *tcdA* are found in approximately 70% of *C. difficile* isolates, while the genes for cytotoxin production are found in all strains of *C. difficile* isolates.

The severity of the disease caused by the bacterium is conferred by the presence of the genes (*cdtA* and *cdtB* genes), which code for the production of binary toxins (41). The pathogenicity of *C. difficile* is mainly due to the presence of two large protein toxins (toxin A and toxin B) and the fully described binary toxin (42). Toxin A is a potent enterotoxin that causes the accumulation of fluid in the gut, is cytotoxic to cells in tissue culture, and is perhaps lethal to experimental animals (43). Toxin B has been reported to be a 1,000-fold more potent cytotoxin than toxin A (but is not an enterotoxin) in original animal studies. Still, there are reports that some strains produce toxin B and not toxin A, which causes severe diarrhea in humans (44). More so, the reports of *C. difficile* strains are toxin A–negative and toxin B–positive, which have been associated with human diseases described in certain epidemics around the world (45). The variation in the findings in this study could be a function of geographical differences and environmental conditions with respect to cultural methods and methods used for the analysis of the bacterium.

The findings in this study are consistent with the reports from literature, which have it that *C. difficile* from humans or animals are commonly resistant to macrolides (erythromycin) and tetracyclines as well as fluoroquinolones (moxifloxacin) and lincosamides (clindamycin) (36). Also, consistent with the finding in this study was the report by Knetsch et., (46), who opined that the global *C. difficile* population contained a broad array of antibiotic-resistance genes encoding resistance to tetracycline and erythromycin.

Our study provides baseline data for the prevalence of *C. difficile* in clinical samples in southeast Nigeria in the following ways; (i) chances of severe infections for this pathogen in the studied location would likely to be there even were low prevalence and non-possession of genes for binary toxin production in most of the isolates in the study was reported; (ii) the study has re-enacted the idea that the alcohol shock method for isolation of *C. difficile* is more effective than the heat-shock method; and (iii) pathogenic *C. difficile* isolates in the studied location were multi-drug resistant.

Conclusion:

This study evaluated the prevalence, molecular characteristics and antibiotic susceptibility patterns of *C. difficile* in southeastern Nigeria, revealing high sensitivity to amoxicillin-clavulanic acid, gentamicin and meropenem, but high resistance to tetracycline and erythromycin, with significant expression of *tcdB* and *tcdA* virulence genes.

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Table 10: Bacterial isolates with their accension numbers

Serial numbers	Bacterial isolates	Accession numbers
1	*Lysinibacillus fusiformis*	PQ462169
2	*Lysinibacillus sphaericus*	PQ462171
3	*Vagococcus humatus*	PQ462172
4	*Enterocloster bolteae*	PQ462174
5	*Clostridium butyricum*	PQ462175
6	*Clostridium butyricum*	PQ462176
7	*Clostridium butyricum*	PQ462177

Availability of data and materials:

The datasets generated and/or analyzed during the study are available on request and the bacterial isolates with accession numbers are as shown in Table 10

Contributions of authors:

EIO did all sample collections, laboratory analysis, writing of the manuscript and arrangement of the manuscript in accordance with the Journal requirements; MCU was the co-supervisor who oversaw the analysis of the research and the manuscript writings; COE was the main supervisor who checked every work done by the corresponding author and the co-supervisor; and OAI assisted with the design of primers to characterize *C. difficile* ensuring the smooth running of the molecular process.

Source of funding:

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Conflict of interest:

Authors declare no conflict of interest.

References:

1. Adegoke, A., Faleye, A., Singh, G., and Stenström, T. Antibiotic-Resistant Superbugs: Assessment of the Interrelationship of Occurrence in Clinical Settings and Environmental Niches. Molecules 2017; 22 (1): 29. https://doi.org/10.3390/molecules22010029.
2. Markantonis, J. E., Fallon, J. T., Madan, R., and Alam, M. Z. *Clostridioides difficile* infection: diagnosis and treatment challenges. Pathogens. 2024; 13: 118. https://doi.org/10.3390/pathogens13020118.
3. Buddle, J. E., and Fagan, R. P. Pathogenicity and virulence of *Clostridioides difficile*. Virulence. 2023; 14 (1): 2150452. doi:10.1080/21505594.2022.2150452.
4. Xiaolu, L., Yizhong, W., Rong, C., et al. Antimicrobial resistance of *Clostridioides difficile* in children from a tertiary pediatric hospital in Shanghai, China. Infect Drug Resist. 2024; 17: 329 - 339. doi:10.2147/IDR.S441312.
5. Centers for Disease Control and Prevention (CDC. Antibiotic Resistance Threats in the United States, CDC, Atlanta, Ga, USA, 2013
6. Parija, S. C. *Clostridium*. In: Textbook of Microbiology and Immunology. Singapore: Springer Nature. 2023: 465-488.
7. Khoder, G., Muhammad, J. S., Mahmoud, I., Soliman, S. S., and Burucoa, C. Prevalence of *Helicobacter pylori* and its associated factors among healthy asymptomatic residents in the United Arab Emirates. Pathogens. 2019; 8(2):44. doi: 10.3390/pathogens8020044
8. Panhwar, A., Abro, R., Kandhro, A., et al. Global Water Mapping, Requirements, and Concerns over Water Quality Shortages. 2022
9. Mondal, S. Prospective observational study to assess patient profile with *Clostridium difficile* colitis. M. Pharm Thesis. 2019 http://20.198.91.3:8080/jspui/handle/123456789/2060
10. Sheen, E., Pan, J., Ho, A., and Triadafilopoulos G. Lower gastrointestinal bleeding. In: Geriatric Gastroenterology. Cham: Springer International Publishing; 2021: 1305-25.
11. Voutsinas, N., Toussie, D., Jacobi, A., Bernheim, A., and Chung, M. Incidental CT findings in the lungs in COVID-19 patients presenting with abdominal pain. Clin Imaging. 2020; 67: 1-4. doi: 10.1016/j.clinimag.2020.05.021
12. Jacek, C., Mirosław, D., Hanna, P., et al. Outbreak of *Clostridium difficile* infection in Europe and North America. Eur J Clin Microbiol Infect Dis. 2019; 38 (7): 1211-1221. doi: 10.1007/s10096-019-03539-6.
13. Doyle, E. M. *Clostridium difficile* as a Risk Associated with Animal Sources. *FRI Food Safety Reviews.* Food Research Institute, University of Wisconsin. 2013: 1-18.
14. Naing, L., Nordin, R. B., Rahman, H. A., and Naing, Y. T. Sample size calculation for prevalence studies using Scalex and ScalaR calculators. BMC Med Res Methodol. 2022; 22:209. doi: 10.1186/s12874-022-01694-7
15. Bridson, E. Y. The OXOID Manual. 9th edn. Basingstoke, UK: Oxoid Limited; 2006.
16. Willey, J., Sherwood, L., and Woolverton, C. Prescott's Microbiology. 7th edn. New York: McGraw-Hill; 2008.
17. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne (PA): CLSI; 2020: 332.
18. Chitanand, M. P., Kadam, T. A., Gyananath, G., Totewad, N. D., and Balhal, D. K. Multiple antibiotic resistance indexing of coliforms to identify high-risk contamination sites in aquatic environment. Indian J Microbiol. 2010; 50: 216–220. doi: 10.1007/s12088-010-0042-9
19. Chakravorty, D., Helb, D., Burday, M., Connell, N., and Alland, D. Use of an integrated MS-multiplexed MS/MS data acquisition strategy for high-coverage peptide mapping studies. Rapid Commun Mass Spectrom. 2007; 21 (5): 730-744. doi: 10.1002/rcm.2888.
20. Thermo Fisher Scientific. BigDye™ Terminator v3.1 Cycle Sequencing Kit: Protocols and Applications. Thermo Fisher Scientific; 2020. https://www.thermofisher.com
21. Amparo, G., Yang, A., Ma, P., Salim, C., and

Heiner, C. Direct sequencing from culture. Applied Biosystems; 2015.

22. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32 (5): 1792-1797. doi: 10.1093/nar/gkh340.
23. Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura. K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018; 35 (6): 1547-1549. doi: 10.1093/molbev/msy096
24. Saitou, N., and Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4 (4): 406-425. doi: 10.1093/oxfordjournals.molbev.a040454.
25. Begum, K., Chua, H. C., Alam, M. J., Garey, K. W., and Jo, J. A quantitative PCR to detect non-toxigenic *Clostridioides difficile*. Microbiol Spectr. 2025; 13 (1): e0160824 https://doi.org/10.1128/spectrum.01608-24
26. Thermo Fisher Scientific. Taq DNA polymerase: Protocol and product specifications. Waltham (MA): Thermo Fisher Scientific; 2010. https://tools.thermofisher.com/content/sfs/manuals/taqnative_pps.pdf
27. Bogiel, T., Mikucka, A., and Kanarek, P. Agarose Gel Electrophoresis-Based RAPD-PCR- An Optimization of the Conditions to Rapidly Detect Similarity of Alert Pathogens for the Purpose of Epidemiological Studies. Gels; 2022. http://doi.org/10.3390/gels8120760
28. Microsoft Corporation. Microsoft Excel (Computer Software). Redmond (WA): Microsoft Corporation; 2006.
29. Weese, J. S. *Clostridium difficile* in food-innocent bystander or serious threat? Clin Microbiol Infect. 2010;16(1):3–10. doi:10.1111/j.1469-0691.2009.03108.x.
30. Warriner, K., Xu, C., Habash, M., Sultan, S., and Weese, S. Dissemination of *Clostridium difficile* in food and the environment: Significant sources of *C. difficile* community-acquired infection? J Appl Microbiol. 2017;122(3):542-553. https://doi.org/10.1111/jam.13338.
31. Weese, J. S., Finley, R., Reid-Smith, R. R., Janecko, N., and Rousseau, J. Evaluation of *Clostridium difficile* in dogs and the household environment. Epidemiol Infect. 2010; 138:1100–1104. doi: 10.1017/S0950268809991312.
32. Shehabi, A. A., Abu-Ragheb, H. A., and Allaham, N. A. Prevalence of *Clostridium difficile*-associated diarrhoea among hospitalized Jordanian patients. East Mediterr Health J. 2001; 7: 750-755.
33. Mori, N., and Aoki, Y. Clinical characteristics and risk factors for community-acquired *Clostridium difficile* infection: A retrospective, case-control study in a tertiary care hospital in Japan. J Infect Chemother. 2015; 21 (12): 864-867. https://doi.org/10.1016/j.jiac.2015.09.004.
34. Deane, J., Fouhy, F., Ronan, N., et al. A multicentre analysis of *Clostridium difficile* in persons with cystic fibrosis demonstrates that carriage may be transient and highly variable with respect to strain and level. J Infect. 2021;82 (4): 159-167. https://doi.org/10.1016/j.jinf.2020.12.027.
35. Lawley, T. D., Croucher, N. J., Yu, L. U., et al. Proteomic and genomic characterization of highly infectious *Clostridium difficile* 630 spores. J Bacteriol. 2009; 191: 5377-5386. doi: 10.1128/JB.00597-09
36. Jobstl, M., Heuberger, S., Indra, A., Nepf, R., Kofer, J., and Wagner, M. *Clostridium difficile* in raw products of animal origin. Int J Food Microbiol. 2010; 138 (2): 172–175. doi: 10.1016/j.ijfoodmicro.2009.12.022.
37. Gerding, D. N. Clindamycin, cephalosporins, fluoroquinolones, and *Clostridium difficile*-associated diarrhea: this is an antimicrobial resistance problem. Clin Infect Dis. 2004; 38:646–648. doi: 10.1086/382084
38. Freeman, J., Fawley, W. N., Hobbs, J., et al. Decreasing metronidazole susceptibility in epidemic *Clostridium difficile* strains, abstr. C2-1964. Abstract on 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA. 2009
39. Rodriguez-Palacios, A., Ilic, S., and LeJeune, J. T. *Clostridium difficile* with moxifloxacin/clindamycin resistance in vegetables in Ohio, USA, and prevalence meta-analysis. J Pathog. 2014: 7 doi: 10.1155/2014/158601
40. Shokoohizadeh, L., Alvandi, F., Yadegar, A., Azimirad, M., Hamid-Hashemi, S., and Alikhani, M. A. Frequency of toxin genes and antibiotic resistance pattern of *Clostridioides difficile* isolates in diarrheal samples among hospitalized patients in Hamadan, Iran. Gastroenterol Hepatol Bed Bench. 2021; 14 (2): 165-173.
41. Bacci, S., Mølbak, K., Kjeldsen, M. K., and Olsen, K. E. Binary toxin and death after *Clostridium difficile* infection. Emerg Infect Dis. 2011; 17: 976-982. doi: 10.3201/eid/1706.101483.
42. Chandrasekaran, R., and Lacy, D. B. The role of toxins in *Clostridium difficile* infection. FEMS Microbiol Rev. 2017; 41 (6): 723-750. doi: 10.1093/femsre/fux048.
43. Kuehne, S. A., Cartman, S. T., Heap, J. T., Kelly, M. L., Cockayne, A., and Minton, N. P. The role of toxin A and toxin B in *Clostridium difficile* infection. Nature. 2010; 467: 711-713. https://doi.org/10.1038/nature09397.
44. Barbut, F., Mastrantonio, P., Delmée, M., Brazier, J., Kuijper, E., and Poxton, I. Prospective study of *Clostridium difficile*-associated disease in Europe with phenotypic and genotypic characterization of the isolates. Clin Microbiol Infect. 2007; 13: 1048-1057. doi:10.1111/j.1469-0691.2007.01824.x.
45. Johnson, S., Kent, S. A., and O'Leary, K. J. Fatal pseudomembranous colitis associated with a variant *Clostridium difficile* strain not detected by toxin A immunoassay. Ann Intern Med. 2001; 135: 434-438. doi: 10.7326/0003-4819-135-6-200109180-00012.
46. Knetsch, C. W., Kumar, N., Forster, S. C., et al. Zoonotic transfer of *Clostridium difficile* harboring antimicrobial resistance between farm animals and humans. J Clin Microbiol. 2018; 56 (3): 1384 – 1417. doi: 10.1128/JCM.01384-17.

Anjorin et al. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (2): 164 - 171 https://www.afrjcem.org

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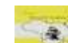

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Original Article **Open Access**

Biochemical evaluation of liver function enzymes in Lassa fever patients

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Abstract:

Background: Lassa fever (LF) is a zoonotic acute viral hemorrhagic disease caused by the Lassa virus (LV). It has a high case fatality rate of about 5,000 deaths in humans from 300,000-500,000 cases each year globally. The high mortality rate from Lassa fever has been associated with abnormal liver enzyme production due to LV infiltration of the liver. However, data are sparse on the different enzymes and their proportions associated with LF. This study aims to evaluate the effect of LF on the levels of selected liver enzymes.
Methodology: This was a case-control epidemiological study of 100 participants comprising 70 participants with LF attending Federal Medical Centre, Owo between May and September 2023, and 30 healthy controls randomly selected within the hospital environs. Demographic information (age and gender) was collected from the participants with data collection form. Venous blood samples were collected from the participants into appropriate sample bottles and centrifuged to separate plasma for laboratory analyses. Lassa fever was confirmed by amplification of LV RNA using reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Specific liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), were assayed from the plasma using Mindray chemistry system with Architect c8000 and Roche Cobas c501 chemistry analyzers. Data were analysed using SPSS version 20.0. software. Mean values were compared using the *t*-test while categorical variables were compared using the Chi-square test. Statistical significance was set at $p<0.05$ with 95% confidence interval.
Results: The results showed that among the study participants, the age group 20-39 years had the highest frequency of LF (37.1%, 26/70), with a slightly higher frequency in the females (51.4%, 36/70), compared to the males (48.6%, 34/70). The mean plasma ALP level of 263.84±50.73 U/L in LF patients was significantly higher than 46.80±1.85 U/L in the controls ($t=35.740$, $p<0.0001$). Similarly, the mean plasma ALT level of 158.96±11.46 U/L in LF patients was significantly higher than 10.67±8.55 U/L in the control ($t=71.405$, $p<0.0001$), and the mean plasma AST level of 283.0±15.71 U/L in LF patients was significantly higher than 19.26±9.02 U/L in the control ($t=105.60$, $p<0.0001$). Elevated plasma levels of ALP, ALT and AST were recorded in 45.7%, 70.0% and 85.7% of LF patients, compared to 0%, 6.7% and 6.7% in the controls respectively ($p<0.0001$).
Conclusion: The study found significantly elevated liver enzymes in LF patients, providing insights into the pathological effects of the LV virus on the liver.

Keywords: Lassa fever, Liver, Enzymes, Biochemical, Evaluation**.**

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Évaluation biochimique des enzymes de la fonction hépatique chez les patients atteints de fièvre de Lassa

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Résumé:

Contexte: La fièvre de Lassa (FL) est une maladie hémorragique virale aiguë zoonotique causée par le virus de Lassa (VL). Son taux de mortalité est élevé, avec environ 5000 décès chez l'homme, sur 300 000 à 500 000 cas chaque année dans le monde. Ce taux élevé de mortalité a été associé à une production anormale d'enzymes hépatiques due à une infiltration hépatique par le VL. Cependant, les données sur les différentes enzymes et leurs proportions associées à la FL sont rares. Cette étude vise à évaluer l'effet de la FL sur les taux de certaines enzymes hépatiques.
Méthodologie: Il s'agissait d'une étude épidémiologique cas-témoins portant sur 100 participants, dont 70 atteints de FL et admis au Centre Médical Fédéral d'Owo entre mai et septembre 2023, et 30 témoins sains sélectionnés aléatoirement dans l'environnement hospitalier. Les informations démographiques (âge et sexe) ont été recueillies auprès des participants à l'aide d'un formulaire de collecte de données. Des échantillons de sang veineux ont été prélevés auprès des participants dans des flacons appropriés, puis centrifugés afin de séparer le plasma en vue d'analyses en laboratoire. La fièvre de Lassa a été confirmée par amplification de l'ARN du VG par RT-PCR. Des enzymes hépatiques spécifiques, dont l'alanine transaminase (ALAT), l'aspartate transaminase (ASAT) et la phosphatase alcaline (PAL), ont été dosées à partir du plasma à l'aide du système de chimie Mindray équipé des analyseurs Architect c8000 et Roche Cobas c501. Les données ont été analysées à l'aide du logiciel SPSS version 20.0. Les valeurs moyennes ont été comparées à l'aide du test t, tandis que les variables catégorielles ont été comparées à l'aide du test du Khi carré. La signification statistique a été fixée à p<0,05 avec un intervalle de confiance à 95%.
Résultats: Les résultats ont montré que parmi les participants à l'étude, le groupe d'âge 20-39 ans avait la fréquence la plus élevée de FL (37,1%, 26/70), avec une fréquence légèrement plus élevée chez les femmes (51,4%, 36/70), par rapport aux hommes (48,6%, 34/70). Le taux plasmatique moyen de PAL de 263,84±50,73 U/L chez les patients atteints de FL était significativement plus élevé que celui de 46,80±1,85 U/L chez les témoins (t=35,740, p<0,0001). Français De même, le taux plasmatique moyen d'ALAT de 158,96±11,46 U/L chez les patients atteints de FL était significativement plus élevé que 10,67±8,55 U/L chez le témoin (t=71,405, p<0,0001), et le taux plasmatique moyen d'ASAT de 283,0±15,71 U/L chez les patients atteints de FL était significativement plus élevé que 19,26±9,02 U/L chez le témoin (t=105,60, p<0,0001). Des taux plasmatiques élevés d'ALP, d'ALAT et d'ASAT ont été enregistrés chez 45,7%, 70,0% et 85,7% des patients atteints de FL, contre 0%, 6,7% et 6,7% chez les témoins respectivement (p<0,0001).
Conclusion: L'étude a révélé une élévation significative des enzymes hépatiques chez les patients atteints de FL, ce qui permet de mieux comprendre les effets pathologiques du virus LV sur le foie.

Mots-clés: Fièvre de Lassa, Foie, Enzymes, Biochimie, Évaluation

Introduction:

Lassa fever (LF) presents as an acute viral hemorrhagic illness and was first identified in 1969 in the town of Lassa, located in Borno State, Nigeria, specifically within the Yedseram River valley at the southern end of Lake Chad (1). The disease is widespread in West African nations, with 300,000 - 500,000 cases each year, resulting in approximately 5,000 fatalities (2). Outbreaks of Lassa fever are endemic to West Africa, specifically occurring in Nigeria, Liberia, Sierra Leone, Guinea, and the Central African Republic (3). Emerging evidence suggests that human infections occur in the Democratic Republic of Congo, Mali, and Senegal. Additionally, cases have been exported to other countries by infected travelers (3).

The causative agent is the Lassa virus (LV), an RNA virus belonging to the Arenaviridae family. The African soft-furred rat (*Mastomys natalensis*) serves as the natural reservoir for Lassa fever. Widely distributed across West Africa, this rodent species closely interacts with humans. The virus is excreted in the urine of *Mastomys* spp and contamination of human food represents a probable route of transmission (4). Human-to-human transmission of the virus can occur through contact with bodily fluids in healthcare settings, leading to nosocomial epidemics with case fatality rates (CFR) as high as 65% (5).

Illness in humans develops within 3 weeks after infection with LV (4). The early features of LF are general and might include fever, fatigue, headache, throat discomfort, muscle pain, cough, chest pain, abdominal pain, nausea, vomiting, and diarrhoea (6). In most cases, symptoms are mild, however, severe illness complicated by abnormal bleeding, generalized edema, respiratory distress, hypotension, proteinuria, transaminitis, deafness, encephalopathy, and/or hypotension develops in approximately 20% of cases (1).

Although the overall mortality rate for LF is minimal, it reaches 15 to 20% for hospitalized patients (7). Higher fatality rates have been reported during outbreaks and among pregnant women, particularly in the third trimester of pregnancy (8). Furthermore, the liver is adversely affected by the LV and this may lead to a rise in the production of liver enzymes. Lassa fever on the other hand has a prevalence of 5% in Nigeria and up to 15% in

the study area, as it has been endemic to the region in previous years (9). It is seen that LF which adversely affects the functionality of the liver may lead to an abnormal production of liver enzymes, leading to increased mortality.

Diagnostic procedures are crucial in managing LF outbreak as they enable early identification, facilitating prompt antiviral treatment and minimizing illness and death. They also aid in tracing community contacts and providing an accurate assessment of the epidemic (10). Based on the classification of the virus, the National Institute of Allergy and Infectious Diseases has designated the LV as a 'Category A' pathogen, highlighting its significant potential for public health impact. To establish a diagnosis, laboratory testing is crucial because clinical symptoms alone are inadequate for distinguishing LF from other febrile illnesses (11).

A suspect case must be promptly excluded or confirmed to enable suitable case management, which may involve treatment, isolation protocols or contact tracing. LF is most often diagnosed by serology using enzyme-linked immunosorbent assay (ELISA), which detect IgM and IgG antibodies as well as LV antigen. During the initial days of illness, only a subset of patients exhibits detectable IgM and IgG antibodies. Interestingly, patients with fatal LF may not produce antibodies at all (12). Therefore, reverse transcription-polymerase chain reaction (RT-PCR) serves as a valuable and prompt diagnostic method for LF, and RT-PCR is applicable during the initial phase of the disease.

Nevertheless, in response to the recommendation of the World Health Organization (WHO) for early diagnostic tests for LF (6) and recognizing LV as a high-priority pathogen with epidemic potential within the WHO Research and Development framework (6), this study was necessary. Furthermore, the recent high prevalence of LF in Nigeria according to the 2024 data from the Nigeria Center for Disease Control (NCDC), has resulted in its increased unpredictable clinical manifestations especially in asymptomatic patients thus justifying the need to study the potential effect of the virus on vital organs of the body such as the liver (13). The present study aims to evaluate the effects of LF on the functionality of the liver by assay of selected liver enzymes in the blood.

Materials and method:

Study area:

The study was conducted at the Molecular Laboratory of the Federal Medical Center, Owo, Ondo State, Nigeria, a city located on the southern edge of the Yoruba Hills that serves as a crossroads for routes from nearby towns (Fig 1). Owo had a population of over 220,000 as of the 2006 census, primarily inhabited by the Yoruba ethnic group with influences from urbanization and industrialization.

Fig 1 : Map of Ondo State, Nigeria showing the location of the study area (https://soluap.com/ondo-map-showing-local-governmen-areas/)

Ethical consideration:

Ethical clearance for this study was obtained from the Ethical Review Board of Federal Medical Center, Owo after a successful review and consideration with identification number FMC/OW/380/VOLCLXX XVII/169. In addition, informed consent and/or assent were obtained from the study participants as applicable. The study adhered to the principles outlined in the 1964 Helsinki Declaration and its current amendments, or equivalent ethical standards.

Study design:

This study was a case-control design with LF patients as the case group and healthy persons as the control group.

Sampling technique:

A randomized sampling technique was used to select 70 LF patients, who were matched with 30 controls by age and gender. The clinical characteristics of the controls were assessed, including their health status and presence of other diseases.

Sample size determination:

The sample size formula proposed by Pourhoseingholi et al., (14) was used to calculate the sample size for the LF patients (case) for the study, given as $N = t^2p(1-p)/m^2$, where N = sample size, p = prevalence rate (5%= 0.05), m = margin of error (=0.05), and t = level confidence at 95% (=1.96).

Applying the formula, a sample size of 73 was calculated using a prevalence rate of 5% (p=0.05) for LF in Ondo State, Nigeria (9). However, only 70 LF patients were recruited as case and 30 healthy persons as control.

Inclusion and exclusion criteria:

The inclusion criteria for the case are patients with symptoms associated with LF such as fever, malaise, bleeding, and other systemic symptoms, with laboratory confirmation of LF by RT-PCR or serological evidence of recent infection. Individuals with existing comorbidities that could influence the outcomes or clinical manifestations of LF were excluded.

Data collection:

Primary data were collected using structured questionnaire and other data were collected from the LF surveillance register in the study area, with some participants completing the questionnaire independently and for some, face-to-face interviews were conducted while adhering to safety guidelines.

Sample Collection and processing:

About 4 ml of venous blood was collected from each participant into lithium heparin bottle for enzyme analysis. With the sample being highly infectious, personal protective equipment (PPE) and triple packaging were used for prevention and containment. The blood samples were centrifuged and plasma separated. The plasma was initially stored at -20°C before being transferred to -80°C for long-term storage. For LV detection, blood was collected into EDTA bottle and centrifuged at 1500 rpm for 10 minutes**.** The plasma was separated and labelled in a sample tube.

Detection of LV RNA:

Inactivation procedures:

The AVL tubes containing lysis buffer and other constituents were used for inactivation of samples. Approximately 70µL of the samples were added to AVL tubes, mixed and allowed to stand for 10mins. About 560µL of absolute ethanol was then added to the AVL tubes with pipette tips being changed at each time. The AVL tubes were decontaminated for RNA extraction (9).

RNA extraction:

Inactivated samples were transferred from the tubes to freshly labelled spin columns. The columns were subsequently centrifuged for 1 minute at 8000 rpm and the collection tubes were emptied and replaced. Subsequently, a series of wash steps were performed; 500µL of AW1 was added, followed by centrifugation at 8000 rpm; 500µL of AW2 was added, followed by centrifugation for 3 minutes at 14000 rpm. This process was repeated for AW2 and 14000 rpm centrifugation. Finally, the column was centrifuged at 14000 rpm for 10 minutes to dry the RNA. The spin column was placed on the elution tube, and 60µL of AVE was added. After incubating for 1 min, the column was centrifuged at 8000rpm for 1min to collect the trapped RNA (9).

Master mixing and PCR:

The RealStar® Lassa Virus RT-PCR Kit 2.0 containing 2 different RT-PCR assays with 48 reactions each was used for the assay. This contains two different positive controls; one for GPC gene-specific amplification 0.5µM primers OWS-1-fwd (GCG CAC CGG GGA TCC TAG GC), and one for the L gene-specific amplification 0.5µM primer OWS-1000-rev (AGC ATG TCA CAA AAY TCY TCA TCA TG). About 20µL each was pipetted into Master G reagent into the G labelled tubes and Master L reagent into the L labelled tubes. 1µL of internal control was added into the tubes. 10µL of the sample (extracted RNA) from the elution tube was added. Positive control was added to one tube of both the G and L tubes.

The RT-PCR mixture was subsequently incubated at 50°C for 30 minutes for reverse transcription, followed by 45 cycles in a thermocycler (Eppendorf, Germany), which included the following steps; denaturation at 95°C for 15 min, activation at 95°C for 30 sec, ann-

ealing at 55°C for 30 sec, elongation at 72°C for 30 sec, and extension at 30°C for 30 sec (15).

Liver enzymes analytical methods:

The levels of the 3 enzymes, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were assayed in the plasma using the Mindray Chemistry system, which utilizes the Architect c8000 and Roche Cobas c501 chemistry analyzers (16).

Statistical analysis:

Data were analysed using SPSS version 20.0 software. Continuous variables (e. g. mean) were compared using a *t*-test, while categorical variables (e. g. age and gender) were compared using the Chi-square test, with Odds ratio (OR) and confidence interval (CI). Statistical significance was set at $p<0.05$ with confidence level of 95%.

Results:

Demographic characteristics of participants:

Table 1 shows the mean age of the LF patients (case) as 38.05±1.83 years while that of the controls was 42.03±1.92 years. There were 36 (48.6%) males and 34 (51.4%) females in the case group, and 16 (53.3%) males and 14 (46.7%) females in the control group.

The age group 20-39 years had the highest frequency of LF (case) with 37.1% (26/70) and the age group 80-99 years had the lowest frequency of 2.9% (2/70). For the control group, the age group 40-59 years were the most frequently selected control (43.3%, 13/30) while the age group 20-39 years were the least frequently selected control (10.0%, 3/30).

Comparison of plasma alkaline phosphatase (ALP) levels between LF patients (case) and controls:

The mean ALP level in LF patients (case) is 263.84±50.73 U/L while that of the control is 46.80±1.85 U/L. A total of 32 out of the 70 LF patients (45.7%) had elevated (above normal value) ALP level, while all (100.0%) the controls had normal ALP levels (OR= 51.54, $p<0.0001$) (Table 2).

Table 1: Demographic characteristics of the study participants (case and control) at Federal Medical Center, Owo, Nigeria

LF patients (n=70)			Control patients (n=30)		
Variable	**Frequency**	**Percentage**	**Variable**	**Frequency**	**Percentage**
Age group (years)			**Age group (years)**		
0-19	10	14.3	0-19		
20-39	26	37.1	20-39	7	23.3
40-59	25	35.7	40-59	3	10.0
60-79	7	10.0	60-79	13	43.3
80-99	2	2.9		7	23.3
Mean age (±SD)	38.05±1.83		**Mean age (±SD)**	42.03±1.92	
Gender			**Gender**		
Male	34	48.6	Male	14	46.7
Female	36	51.4	Female	16	53.3

SD = Standard deviation

Table 2: Comparison of plasma ALP levels between LF (case) and control patients at Federal Medical Center, Owo, Nigeria

ALP level	LF patients (n=70)		Control patients (n=30)		OR (95% CI)	*p* value
	Frequency	**Percentage**	**Frequency**	**Percentage**		
Normal value	38	54.3	30	100	51.54 (3.027 - 875.96)	<0.0001*
Above normal	32	45.7	0	0		
Mean ±SD (U/L)	263.84±50.73		46.80±1.85		*t* = 35.740	<0.0001*

ALP = Alkaline phosphatase; LF=Lassa fever; OR=Odd ratio; CI=confidence interval; SD = Standard deviation; * = statistically significant at $p<0.05$

Table 3: Comparison of plasma ALT levels between LF (case) and control patients at Federal Medical Center, Owo, Nigeria

ALT level	LF patients (n=70)		Control patients (n=30)		OR (95% CI)	*p* value
	Frequency	Percentage	Frequency	Percentage		
Normal	21	30.0	28	93.3	32.667 (7.121 - 149.85)	<0.0001*
Above Normal	49	70.0	2	6.7		
Mean ± SD (U/L)	158.96±11.46		10.67±8.55		*t* = 71.405	<0.0001*

ALT = Alanine transaminase; LF=Lassa fever; OR=Odd ratio; CI=confidence interval; SD = Standard deviation; * = statistically significant at $p<0.05$

Table 4: Comparison of plasma AST levels between LF (case) and control patients at Federal Medical Center, Owo, Nigeria

AST level	LF patients (n=70)		Control patients (n=30)		OR (95% CI)	*p* value
	Frequency	Percentage	Frequency	Percentage		
Normal	10	14.3	28	93.3	84.00 (17.242 - 409.23)	<0.0001*
Above Normal	60	85.7	2	6.7		
Mean ± SD (U/L)	283.0±15.71		19.26±9.02		*t* = 105.60	<0.0001*

AST = Aspartate transaminase; LF=Lassa fever; OR=Odd ratio; CI=confidence interval; SD = Standard deviation; * = statistically significant at $p<0.05$

Comparison of plasma alanine transaminase (ALT) levels between LF (case) and control patients:

The mean ALT level in LF patients (case) is 158.96±11.46 U/L while that of the control is 10.67±8.55 U/L. A total of 49/70 (70.0%) LF patients had higher than the normal ALT levels, compared to only 2/30 (6.7%) control patients (OR=32.667, $p<0.0001$) (Table 3).

Comparison of plasma aspartate transaminase (AST) levels between LF (case) and control patients:

The mean AST level in LF patients (case) is 158.96±11.46 U/L while that of the control is 10.67±8.55 U/L. A total of 60/70 (85.7%) LF patients had above-normal AST levels compared to only 2/30 (6.7%) control patients (OR=84.00, $p<0.0001$) (Table 4).

Discussion:

Despite the public outcry and efforts of international public health agencies to control the widespread LF characterized by high morbidity and mortality in several areas, the disease continues to spread in different communities from time to time. This highlights the need for continuous integrated surveillance and indicators including biochemical parameters for prompt detection of the disease in LF patients. In this study, the highest frequency LF of 37.1% was recorded within the age group 20-39 years which shows that the youth are the most vulnerable because they are usually exposed to predisposing factors such as games hunting, farming, healthcare delivery, and other cultural activities. This is consistent with a similar report by Grace et al*.,* (17) of the predominant age group affected being 21-30 years. Additionally, comparable findings have been reported in a study conducted in 20 States in Nigeria by Elimian et al., (18) in which the most vulnerable group is between the age group 31-40 years. Nevertheless, another study conducted in Nigeria (19) reported that individuals in age group 15-34 years were the most impacted, with the next highest prevalence observed among those in the age group 35-54 years. It is noteworthy that those mostly affected by LF are the economically productive age group, which aligns with the previous studies conducted in Jos and the neighbouring Bauchi State in Nigeria (20,21).

However, in our study, the frequency was lower among other age groups, with the lowest frequency of 2.9% recorded in the age group 80-99 years. This could be partly attributed to their limited exposure to the LV and social activities (20). The observed variability in findings may be linked to differences in study location, timeframe, and population characteristics. Additionally, our study revealed a slightly higher frequency among female compared to male. This disparity could be partly attributed to the fact that females are primarily responsible for household chores, including cleaning, cooking, and food storage, as well as their involvement in agricultural activities such as farming and crop harvesting (22).

It is noteworthy that these activities increase the likelihood of exposure to infected rodents and their excreta, as they often involve handling and storing food items that may be contaminated. This finding is consistent with a previous study conducted in Sierra Leone, where females were mostly affected by LF (23). The results of our study align with the

broader trends observed across West Africa, further confirming that LF disproportionately affects the female population in endemic regions (24). These findings have important implications for targeting prevention and control efforts to address the underlying social, behavioural, and biological factors driving this gender disparity (24, 25).

Furthermore, in this study, more than 70% of the LF patients had elevated liver enzymes, which agrees with the results obtained from a previous study by Hassan et al*.,* (9) that LF, an acute viral hemorrhagic illness, greatly increased the levels of liver enzymes, specifically ALT, AST, and ALP, in the body. About 45.7% of LF patients had above-normal levels of ALP. This may be attributed to the direct viral-induced damage to the liver, which causes the release of this enzyme into the bloodstream. However, specific studies directly linking LF to ALP elevation remain limited. Further research is needed to understand the mechanisms and clinical implications of ALP changes in LF patients (26).

Moreover, 70.0% of the LF patients had above normal ALT levels compared to 2.0% of the controls. This may be partly due to the high levels of the ALT enzyme, which is primarily found in the liver, with lower concentrations observed in the heart, kidneys, skeletal muscle, pancreas, lung tissues, and spleen. The results of our study align with the findings of Gowda et al*.,* (27) who reported that the normal concentration levels of ALT in the blood are usually low, however, when liver damage occurs, the liver releases more ALT into the blood causing the level to rise. Measurement of ALT is crucial in diagnosing hepatic damage and various liver diseases, including viral hepatitis and cirrhosis (28).

Alanine transaminase is frequently assessed alongside AST, an enzyme that is present in cells throughout the body and is most abundant in the liver and the heart, and to a lesser extent, in the muscle tissues and kidneys. In healthy individuals, blood AST levels remain low; however, when the liver or muscles sustain injury, AST is released into the bloodstream (29). Our study showed 85.7% of the LF patients had above-normal AST levels, compared to only 2% of the negative controls. The presence of liver damage, as evidenced by specific clinical or laboratory findings suggests LF. Our study is limited by the inability to obtain the cycle threshold (*ct*) value that is useful in classifying the increase of the viral load base. This is recommended for further study.

Conclusion:

This study reports significant elevation in liver enzymes (ALT, AST, and ALP) in patients with LF. This finding suggests that LF directly contributes to the increased production of liver enzymes, indicating potential liver damage. Adequate therapy will reduce the effects of LV on the liver and the observed elevated levels of liver enzymes (ALT, AST, and ALP) in LF patients.

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Contributions of authors:

EJ and GBO collected blood samples and performed sample analysis. HAO conceived and designed the study. OTE, HAO, SWO, ASO, LAO, EJ, GBO and AAA contributed to the manuscript writing. OTE, HAO, SWO, ASO, and AAA performed data analysis. The manuscript was finally edited by AAA, HAO, SWO, and ASO. All authors approved the final reviewed draft of the manuscript for publication.

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References:

1. Fram, J. D., Baldwin, J. J., Gocke, D. J., and Troup, J. M. Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. 1970. https://doi.org/10.4269/ajtmh.1970.19.670
2. Ogbu, O., Ajuluchukwu, E., and Uneke C. Lassa fever in West African sub-region: an overview. J Vect Borne Dis. 2007; 44 (1) :1. https://pubmed.ncbi.nlm.nih.gov/17378212/
3. Atkin, S., Anaraki, S., Gothard, P., et al. The first case of Lassa fever imported from Mali to the United Kingdom, February 2009. EuroSurveill. 2009; 14 (10): 19145. doi: 10.2807/ese.14.10.19145-en
4. Kakaī, C. G., Okunromade, O. F., Dan-Nwafor, C. C., et al. Improving cross-border preparedness and response: lessons learned from 3 Lassa fever outbreaks across Benin, Nigeria, and Togo, 2017-2019. Health Security. 2020; 18 (S1): S-105 - S-112. doi: 10.1089/hs.2019.0080
5. Dan-Nwafor, C. C., Ipadeola, O., Smout, E., et al. A cluster of nosocomial Lassa fever cases in a tertiary health facility in Nigeria: Description and lessons learned, 2018. Int J Infect Dis. 2019; 83: 88-94. https://doi.org/10.1016/j.ijid.2019.03.030
6. Salami, K., Gsell, P-S., Olayinka, A., et al. Meeting report: WHO consultation on accelerating Lassa fever vaccine development in endemic countries, Dakar, 10–11 September 2019. Vaccine. 2020; 38 (26): 4135-4141. https://doi.org/10.1016/j.vaccine.2020.01.017

7. Siegel, J. D., Rhinehart, E., Jackson, M., and Chiarello, L. Committee HCICPA. 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Contr. 2007; 35 (10): S65. doi: 10.1016/j.ajic.2007.10.007
8. Shaffer, J. G., Grant, D. S., Schieffelin, J. S., et al. Lassa fever in post-conflict Sierra Leone. PLoS Negl Trop Dis. 2014; 8 (3): e2748. https://doi.org/10.1371/journal.pntd.0002748
9. Hassan, A. O., Onyeaghala, E. O., Etafo, J., Gbenga-Ayeni, B. O., and Obeagu, E. Comparative analysis of the impact of two variations of Qiagen extraction procedure on PCR amplification in Lassa fever diagnosis in Owo. Madonna University Journal of Medicine and Health Sciences. 2022; 2 (3): 85-101.
10. Hamblion, E., Raftery, P., Wendland, A., et al. The challenges of detecting and responding to a Lassa fever outbreak in an Ebola-affected setting. Int J Infect Dis. 2018; 66: 65-73. https://doi.org/10.1016/j.ijid.2017.11.007
11. Fukushi, S., Tan, H., Yoshikawa, T., Saijo, M., and Morikawa, S. Serological assays based on recombinant viral proteins for the diagnosis of arenavirus hemorrhagic fevers. Viruses. 2012; 4 (10): 2097-2114. doi: 10.3390/v4102097
12. Johnson, D. M. Development of rationally designed live attenuated vaccines for Lassa fever and Venezuelan equine encephalitis. 2020. https://doi.org/10.18297/etd/3393
13. Adesola, R. O., Idris, I., Bakre, A. A., Arthur, J. F., and D'Souza, J. N. Challenges Associated With Re-Emergence of Lassa Fever in Nigeria: An Exploratory Study of Epidemiology, Phylogenomics, and Recommendations Toward Its Eradication. Health Sci Rep. 2024; 7 (12): e70225. doi: 10.1002/hsr2.70225
14. Pourhoseingholi, M. A, Vahedi, M., Rahimzadeh, M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench. 2013; 6 (1): 14 - 17.
15. Boom, R., and Sol, C. J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 2006; 28: 495-503 doi 10.1128/jcm.28.3.495-503.1990
16. Adeyemi, O. T., Osilesi, O., Adebawo, O. O., Onajobi, F. D., Oyedemi, S. O., and Afolayan, A. Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) Activities in selected tissues of rats fed on processed atlantic horse mackerel (*Trachurus trachurus*). Adv Biosci Biotechnol. 2015; 6 (03): 139. doi: 10.4236/abb.2015.63014
17. Grace, J-UA., Egoh, I. J., and Udensi, N. Epidemiological trends of Lassa fever in Nigeria from 2015-2021: A review. Ther Adv Infect Dis 2021; 8: 20499361211058252. doi: 10.1177/20499361211058252
18. Elimian, K., Ochu, C., Ilori, E., et al. Descriptive epidemiology of coronavirus disease 2019 in Nigeria, 27 February–6 June 2020. Epidemiol Infect. 2020; 148: e208. doi: 10.11604/JIEPH.supp.2021.4.2.1155]
19. Buba, M. I., Dalhat, M. M., Nguku, P. M., et al. Mortality among confirmed Lassa fever cases during the 2015–2016 outbreak in Nigeria. Am J Publ Health. 2018; 108 (2): 262-264. doi: 10.2105/AJPH.2017.304186
20. Abdulkarim, M. A., Babale, S. M., Umeokonkwo, C. D., et al. Epidemiology of Lassa fever and factors associated with deaths, Bauchi State, Nigeria, 2015–2018. Emerg Infect Dis. 2020; 26 (4): 799. doi: 10.3201/eid2604.190678
21. Chollo, S,. Osawe, S., Lar, P., Egah, D., Mamman, I., and Abimiku, A. l. Analysis of reported cases of Lassa Fever in Plateau State and the need for strategic action plan. Int J Trop Dis Health. 2016; 13 (2): 1-7. doi: 10.9734/IJTDH/2016/20568
22. Pierotti, R. S., Friedson-Ridenour, S., and Olayiwola, O. Women farm what they can manage: How time constraints affect the quantity and quality of labor for married women's agricultural production in southwestern Nigeria. World Dev. 2022; 152: 105800. https://doi.org/10.1016/j.worlddev.2021.105800
23. Kassim Kamara et al . Analysis of national Lassa fever surveillance data, Sierra Leone, 2012 – 2018. Journal of Interventional Epidemiology and Public Health. 2023;6:7. doi: 10.37432/jieph.2023.6.2.79
24. Ekechi, H. U., Ibeneme, C., Ogunniyi, B., et al. Factors associated with a confirmed Lassa fever outbreak in Eguare community of Esan West, Edo State, Nigeria: January-March, 2019. J Interval Epidemiol Publ Health. 2020; 3 (1): 1-12. doi 10.14202/IJOH.2021.128-134
25. Olugasa, B. O., and Dogba, J. B. Mapping of Lassa fever cases in post-conflict Liberia, 2008-2012: A descriptive and categorical analysis of age, gender and seasonal pattern. Ann Afr Med. 2015; 14 (2): 120-122. doi 10.4103/1596-3519.149890
26. Mantri, N., Miranda, J., Jyala, A., Badipatla, K. R., and Nayudu, S. K. 2619 Persistently Elevated Intestinal Alkaline Phosphatase in an Individual With AB Blood Group: A Rare Presentation. Am J Gastroenterol. 2019; 114: S1441-S1442. doi: 10.14309/01.ajg.0000600008.33110.22
27. Gowda, S., Desai, P. B., Hull, V. V., Avinash, A. K., Vernekar, S. N., and Kulkarni, S. S: A review on laboratory liver function tests. Pan Afr Med J. 2009; 3.
28. Kasarala, G., and Tillmann, H. L. Standard liver tests. Clin Liver Dis. 2016 ; 8 (1): 13-18. doi: 10.1002/cld.562
29. Dufour, D. R., Lott, J. A., Nolte, F. S., Gretch, D. R., Koff, R. S., and Seeff, L. B. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Liver Dis. 2000; 46 (12): 2050-2068. doi: 10.4236/tel.2018.810124

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Pathogenicity of filamentous fungi isolated from poultry farms on gastrointestinal system of day-old chicks in Anambra State, Nigeria

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Abstract:

Background: The pervasive presence of filamentous fungi in poultry farming environments has a profound impact on the bird health, leading to reduced growth rates, increased mortality, and decreased egg production. The objective of this study was to use an avian model to determine the pathogenicity of some filamentous fungal isolates from litter, feed, and fecal samples on day-old birds.
Methodology: A total of 300 environmental samples (feed, litter, and droppings) were collected from 10 selected poultry farms in six local government areas (LGAs) in Anambra State, and cultured for fungi isolation by phenotypic (macroscopic and microscopic) and genotypic methods. Five of the fungi isolates were used for pathogenicity study on day-old chicks. A total of 25 day-old-chicks, randomly divided into 5 groups of 5 each, were orally infected with 1 ml of the different fungal conidia. A 6th group served as the control and was inoculated with the same volume of phosphate-buffered saline. All the birds were observed twice daily for appearance of clinical signs for 10 days. Histological examination of the intestinal tissues and stomach of infected birds was done. Data were analysed using one-way ANOVA on SPSS version 21.0, with $p<0.05$ considered significant
Results: The pathogenic effects of the 5 fungi isolates (*Curvularia verruculosa, Aspergillus tubingensis, Cunninghamella bertholletiae, Fusarium oxysporum* and *Aspergillus fumigatus*) used for the pathogenicity study on the birds include symptoms such as; (i) reduction in food and water intake, fraying of feathers and loss of reflex within 5 days of exposure to *C. verruculosa,* and (ii) discolored and enlarged bursa of Fabricius, obstructed, dark, and slightly sloughed intestine, reduction in the sizes of livers and gizzards, and swelling of the rectum with exposure to *F. oxysporum* and *A. fumigatus.* Histological examination revealed severe destruction of the intestinal villi, distortion of the intestinal mucosa, and haemorrhage in all the groups of birds exposed to the 5 fungi isolates. Mortality in the groups of birds infected with *C. verruculosa* and *C. bertholletiae* (80.0%, 4/5) was significantly higher ($p<0.001$) than in other groups.
Conclusion: This study showed that the poultry farms were highly laddened with a variety of filamentous fungi, which have a lot of detrimental effects on the health of bird and needs to be controlled through proper hygiene practices and the provision of biosecurity for the birds.

Keywords: Poultry, filamentous fungi, birds, gastrointestinal tract, pathogenicity

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Pathogénicité de champignons filamenteux isolés dans des élevages avicoles sur le système gastro-intestinal de poussins d'un jour dans l'État d'Anambra, au Nigéria

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Résumé:

Contexte: La présence généralisée de champignons filamenteux dans les élevages avicoles a un impact profond sur la santé des oiseaux, entraînant une réduction des taux de croissance, une augmentation de la mortalité et une diminution de la ponte. L'objectif de cette étude était d'utiliser un modèle aviaire pour déterminer la pathogénicité de certains isolats de champignons filamenteux provenant d'échantillons de litière, d'aliments et de matières fécales chez des oiseaux d'un jour.
Méthodologie: Un total de 300 échantillons environnementaux (aliments, litière et fientes) ont été collectés dans 10 fermes avicoles sélectionnées dans six zones de gouvernement local (LGA) de l'État d'Anambra, et cultivés pour l'isolement des champignons par des méthodes phénotypiques (macroscopiques et microscopiques) et génotypiques. Cinq des isolats de champignons ont été utilisés pour des études de pathogénicité sur des poussins d'un jour. Un total de 25 poussins d'un jour, répartis aléatoirement en 5 groupes de 5 chacun, ont été infectés par voie orale avec 1 ml des différentes conidies fongiques. Un 6e groupe a servi de témoin et a été inoculé avec le même volume de solution saline tamponnée au phosphate. Tous les oiseaux ont été observés deux fois par jour pour l'apparition de signes cliniques pendant 10 jours. Un examen histologique des tissus intestinaux et de l'estomac des oiseaux infectés a été effectué. Les données ont été analysées à l'aide d'une ANOVA à un facteur sur SPSS version 21.0, avec $p<0,05$ considéré comme significatif.
Résultats: Les effets pathogènes des 5 isolats de champignons (*Curvularia verruculosa, Aspergillus tubingensis, Cunninghamella bertholletiae, Fusarium oxysporum* et *Aspergillus fumigatus*), utilisés pour l'étude de pathogénicité sur les oiseaux, comprennent des symptômes tels que; (i) réduction de la consommation de nourriture et d'eau, effilochage des plumes et perte de réflexe dans les 5 jours suivant l'exposition à *C. verruculosa*, et (ii) bourse de Fabricius décolorée et élargie, intestin obstrué, sombre et légèrement desquamé, réduction de la taille des foies et des gésiers, et gonflement du rectum avec exposition à *F. oxysporum* et *A. fumigatus*. L'examen histologique a révélé une destruction sévère des villosités intestinales, une distorsion de la muqueuse intestinale et une hémorragie dans tous les groupes d'oiseaux exposés aux 5 isolats de champignons. La mortalité dans les groupes d'oiseaux infectés par *C. verruculosa* et *C. bertholletiae* (80.0%, 4/5) était significativement plus élevée ($p<0,001$) que dans les autres groupes.
Conclusion: Cette étude a montré que les élevages avicoles étaient fortement contaminés par divers champignons filamenteux, qui ont de nombreux effets néfastes sur la santé des oiseaux et doivent être contrôlés par des pratiques d'hygiène appropriées et la mise en place de mesures de biosécurité pour les oiseaux.

Mots-clés: Volaille, champignons filamenteux, oiseaux, tractus gastro-intestinal, pathogénicité

Introduction:

Among the public health issues caused by microbes, fungal diseases are relatively neglected, owing to the low mortality rate of 1.5% globally (1). Filamentous fungi pose significant health risks to poultry, particularly through their insidious effects on the gastrointestinal tract (GIT) of young birds. These fungi impair gut health, reduce nutrient absorption, and increase susceptibility to secondary infections, severely affecting poultry production (2). Fungal pathogens have gained importance due to their wide-ranging impacts, causing infections that vary from superficial to life-threatening conditions in both animals and humans (3). According to the Global Action for Fungal Infections (GAFFI), fungal diseases claim approximately 150 lives per hour worldwide, underscoring the critical need to address their effects in both human and veterinary medicines (4).

Young birds, particularly day-old chicks, are highly vulnerable to fungal infections. Fungi are frequently associated with high morbidity and mortality in these birds, especially when they are exposed to contaminated environments early in life (5). The most common sources of fungal exposure in poultry include settled dust, bio-aerosols derived from soil, dust, mouldy feed, especially processed ones, contaminated litter, droppings and to a lesser extent, the birds themselves (6). These substrates, often found in poultry farms, create conducive environment for fungal growth and the subsequent spread of infection (7).

Fungal genera implicated in poultry farm diseases include *Acremonium*, *Fusarium, Lichtheimia, Aspergillus, Trichoderma, Penicillium*, *Scopulariopsis, Curvularia, Alternaria*, *Histoplasma Microsporium, Trichophyton,* and yeasts such as *Cryptococcus* and *Candida* spp (8,9,10,11). Among these, *Aspergillus, Penicillium* and *Fusarium* are particularly prevalent and have been linked to gastrointestinal infections in poultry (12). The avian gastrointestinal tract (GIT) plays a crucial role in nutrient absorption, and its health is vital for optimal poultry productivity. A healthy and efficiently functioning GIT is essential for sustainable animal production, influencing key performance indicators such as growth rates, feed efficiency, and the quality of meat and eggs (13). However, the GIT is constantly exposed to a range of microorganisms including bacteria, viruses, parasites and fungi. Although fungi make up a small portion (about 0.1%) of the gut microbiome, their presence can disrupt microbial balance, leading to the development of diseases such as mycosis, particularly when immunological defences are compromised (3, 14).

Mycotic infections are often contracted through contaminated feed or water, with fungal spores thriving in poultry litter and droppings, further increasing the risk of infection in farm environments (15). Despite the capacity of the avian immune system to combat many

pathogens, filamentous fungi have evolved to exploit the vulnerabilities of young birds. Their thin skin, poor vascularised air sacs, and lack of sebaceous and sweat glands make birds more susceptible to fungal colonization compared to other vertebrates (16). Once the fungi bypass the physiological barriers and colonize the GIT, they can enter a commensal relationship with the host, sometimes remaining asymptomatic until microbial balance is disrupted (17).

Among the various genera of filamentous fungi, *Aspergillus* species are the most prevalent in poultry environments. Oral exposure to these fungi can lead to gastrointestinal symptoms such as diarrhoea, vomiting, and abdominal pain, and in severe cases, may result in mortality (18). Given the growing recognition of the impact of fungal infections on poultry health and the potential zoonotic risks, it is essential to study these pathogens more closely.

The primary objective of this study is to investigate the pathogenic impact of selected filamentous fungi isolated from litter, feed, and faecal samples in poultry farms located in Anambra State, Nigeria, on the gastrointestinal health of day-old chicks. By understanding the effects of these fungal isolates, the study aims to provide insights into their role in impairing avian GIT health and contribute to developing more effective management strategies for fungal infections in poultry farming.

Materials and method:

Study setting:

This study was conducted on 10 selected poultry farms in 6 local government areas (LGAs) of Anambra State, southeast Nigeria (latitude 6° 20'N and longitude 7° 00'E) from October 2014 to September 2015. The LGAs and farms include Idemili LGA (Apkaka farm in Umuoji, Jospan farm in Nkpor, Osakwe farm in Ogidi, Cyroby farm in Ogidi, Volant farm in Ogidi), Nnewi North LGA (Agroventures farm), Onitsha South LGA (Government farm), Awka South LGA (Takilita farm), Oyi LGA (EM farm), and Aguata L.G.A, (Eagle farm). The farms, housing approximately 227,200 laying birds, were examined. Six out of the 10 farms practiced open (floor) housing system, while the other four practiced closed (cage) housing system (19).

Ethical approval and informed consent:

Ethical approval was obtained from the university ethical committee before conducting the research. The permissions of the farm managements were obtained before sample collection.

Data collection:

A designed questionnaire was used to collect data from the poultry farm workers about the poultry and the clinical conditions of chicks.

Isolation and identification of fungi isolates:

A total of 300 samples (feeds, litters, and droppings) were collected from the selected poultry farms and cultured for isolation and identification of fungi isolates as previously described by Mba et al., (19), which involved comprehensive analyses of macroscopic, microscopic and genetic features of the isolates (20,21,22).

To confirm the identities of the isolates, a subset was sent to Macrogen, Europe (Meibergdreef Amsterdam, Netherlands) for further analysis, the findings of which have been published by Mba et al., (19). Five of the fungal species were selected for pathogenicity study; *Aspergillus tubingensis, Aspergillus fumigatus, Curvularia verruculosa, Fusarium oxysporum*, and *Cunninghamella bertholletiae*.

Pathogenicity testing of the fungal isolates:

Experimental and control chicks:

Thirty-one-day-old chicks were housed in cages (divided into 6 groups of 5 chicks each) in the animal facility of the Zoology Department, University of Nigeria, Nsukka, Nigeria. The chicks were provided with commercial feed and sterile water *ad libitum*. One of the 6 groups served as the control group.

Inoculum preparation:

To prepare the fungal inoculum, the isolates were grown on Sabouraud Dextrose Agar at 25°C for 6 days to induce asexual structures. Fungal spores were harvested by suspending the cultures in phosphate-buffered saline (PBS) containing 0.1% Tween-80. The suspension was filtered through two layers of sterile gauze to remove hyphal fragments. Tween-80 was removed by repeated washing with PBS, and the final spore suspension was adjusted to a concentration of approximately 1×10^6 spores/ml using haemocytometer (23).

Animal inoculation and clinical features:

Each experimental chick was administered 1ml of the fungal inoculum orally. The control group was administered an equivalent volume of PBS. The chicks were monitored twice daily for 10 days as previously described (11,24,25,26) for clinical signs such as diarrhea, vomiting, tremors, loss of balance, lethargy, weight loss, respiratory distress and other morphological and pathological changes including mortality (27,28,29)

Necropsy and histopathological examination:

Any dead bird was immediately subjected to necropsy, and sick birds were taken for further clinical examination. Detailed case histories and observed clinical signs were con-

sidered before conducting necropsy. Gross lesions in organs and tissues were carefully recorded. Tissue samples from the intestines and stomachs of infected birds were collected postmortem and fixed in 10% neutral buffered formalin to prevent tissue autolysis. The fixed tissue samples were processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H & E) following standard protocols (30). Histopathological lesions were examined under a light microscope at 100× magnification. Photomicrographs were captured using a Motic camera, and pathological changes were documented.

Statistical analysis:

Data were analyzed using one-way ANOVA on SPSS version 21.0. Values were considered significant if $p<0.05$.

Results:

Clinical features of experimental (infected) and control birds:

All the birds exposed to fungal isolates exhibited common clinical signs, including diarrhea, vomiting, reduced food and water intake, and weight loss within 5 days of exposure. Additionally, chicks inoculated with *C. verrucalosa*, *A. fumigatus*, and *A. tubingensis* displayed signs of lethargy and dehydration, with mortality rates of 80.0% (4/5) for *C. verrucalosa* and 60.0% (3/5) for both *A. fumigatus* and *A. tubingensis*. Infection with *F. oxysporum* also led to tremors and reduced growth rate, although the mortality rate was relatively low at 20.0% (1/5).

Furthermore, infection with *C. bertholletiae* resulted in high mortality rate of 80.0% (4/5), accompanied by signs of lethargy, dehydration, and frayed feathers. Notably, chicks exposed to *C. verruculosa* also exhibited frayed feathers, loss of balance and reduced reflex within 5 days of exposure. In contrast, the control group administered with an equivalent volume of PBS, which were not exposed to any fungal isolate, exhibited normal, healthy morphological features with no clinical signs and symptoms.

Mortality rate in fungi infected birds:

Fig 1 shows mortality rate of day-old chicks orally exposed to 1 ml inoculum suspension of the different fungal isolates. The group infected with *C. verruculosa* and *C. bertholletiae* recorded the highest mortality rate of 80.0% (4/5) while the group exposed to *F. oxysporum* had the lowest mortality rate of 20% (1/5).

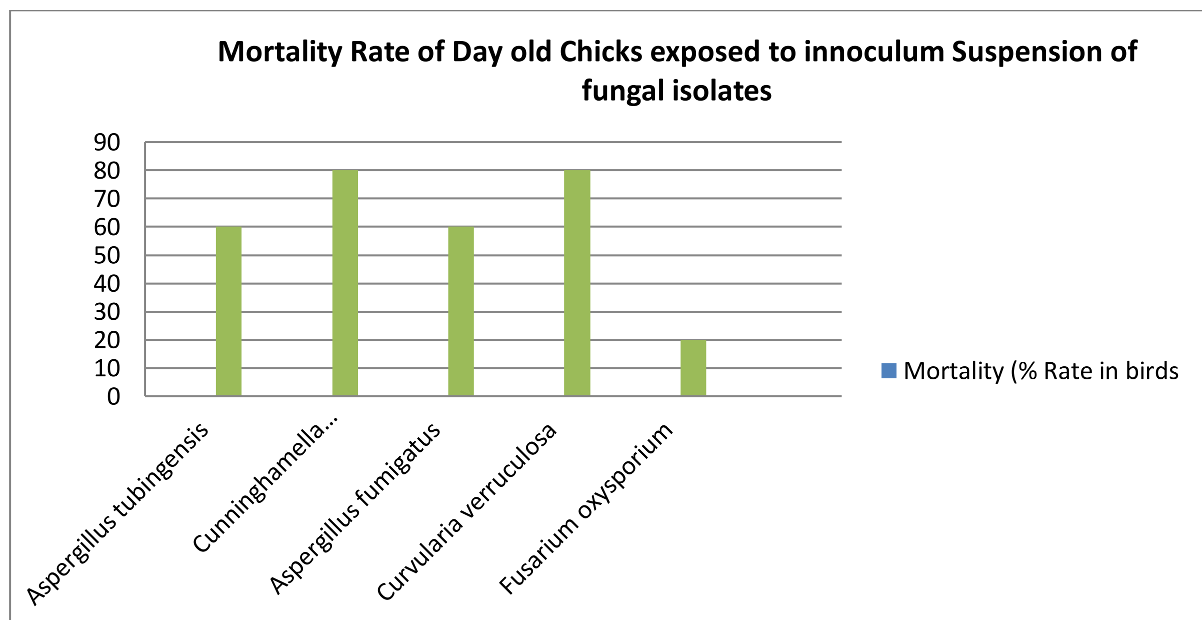
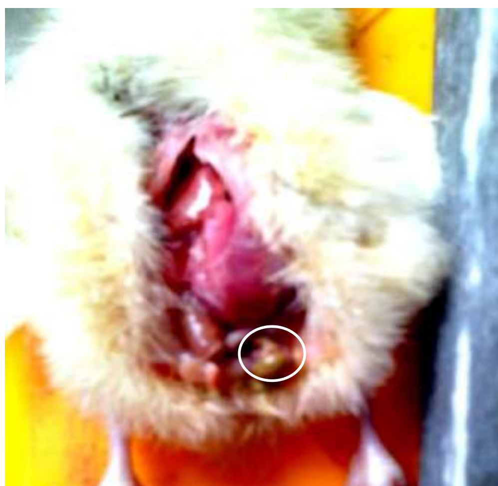


Fig 1: Mortality rate in infected birds

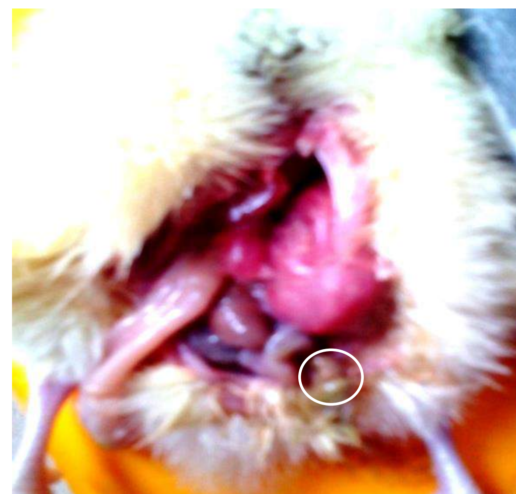
Morphological changes in the intestine of fungi infected birds:

Gross morphological changes were observed in the internal organs of the birds orally infected with different fungal isolates (Plates 1B, 2B, 3 and 4). In contrast, control birds, which were not exposed to any fungal isolate, exhibited normal, healthy morphological features (Plates 1A and 2A).

Plates 1A/2A and 1B/2B: Dissected bird showing various morphological changes in the internal organs, notably discoloured bursa of Fabricius (encircled 2B) and intestinal obstruction (encircled 2B) following oral exposure to *Curvularia verruculosa*



(A)



(B)

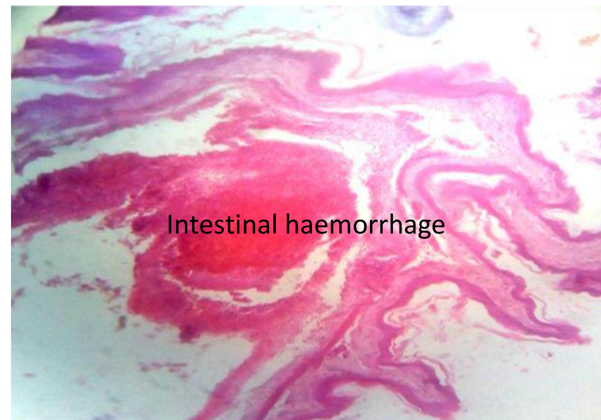
Plate 3: Dissected bird showing different morphological changes in the internal organs especially discoloured bursa of Fabricius (encircled) after oral exposure to; (A) *Aspergillus tubingensis* and (B) *Cunninghamella bertholletiae*

(A) (B)

Plate 4: Dissected bird showing different morphological changes in the internal organs [discoloured bursa of Fabricius (1) and swollen rectum (2)] after oral exposure to; (A) *Fusarium oxysporum*, and (B) *Aspergillus fumigatus*

Histopathological examination of experimental (infected) birds:

Histological analysis of the stomach and intestines of the infected birds revealed pathological changes (Plates 5–8), ranging from severe distortion of the intestinal mucosa to haemorrhages, villi destruction, and ulceration.



(A) (B)

Plate 5: Photomicrograph of orally infected chick intestine with; (A) *Aspergillus tubingensis* showing severe distortion of the mucosa, and (B) *Cunninghamella bertholletiae* showing severe intestinal haemorrhage at the luminal surface (H & E 100x).

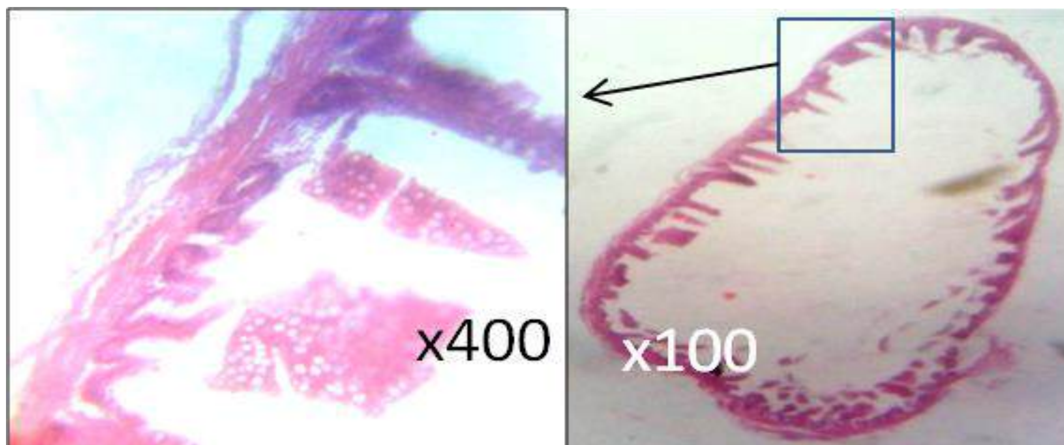
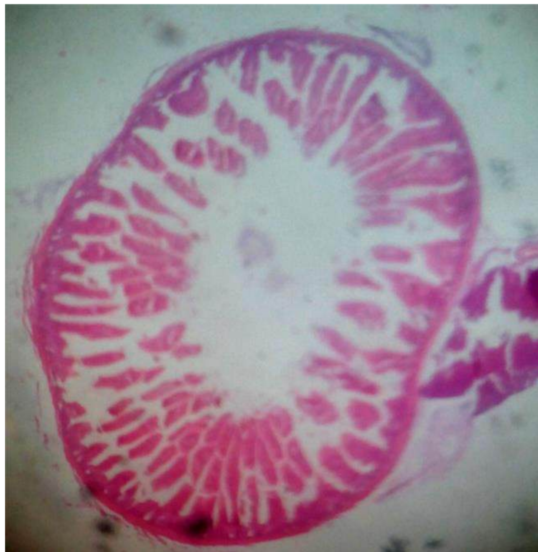
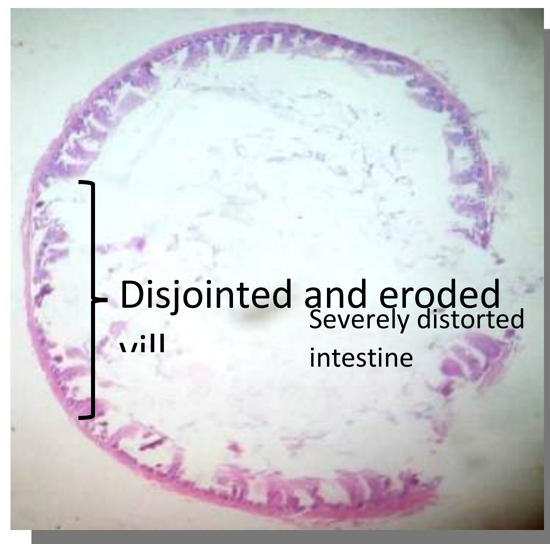


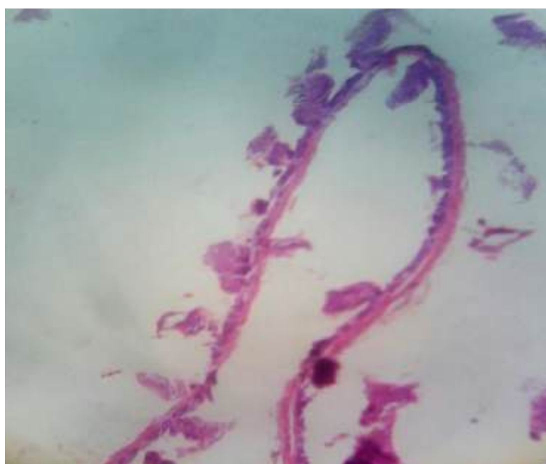
Plate 6: Photomicrograph of chick intestine orally exposed to *Curvularia verruculosa* showing severe destruction and reduction of the fused intestinal villi indicating mucosal atrophy (H & E 100x and X400x)





(A) (B)

Plate 7: Photomicrograph of chick small intestine orally exposed to; (A) *Curvularia verruculosa* showing minor disintegration of the intestinal villi from the base, and (B) *Fusarium oxysporum*, showing severe ulceration through the muscularis mucosa and severe destruction of the villi. (H & E 100x)



(A) (B)

Plate 8: Photomicrograph of *Gallus domesticus* small intestine orally exposed to; (A) *Fusarium oxysporum* showing predeveloped intestinal walls although the mucosal walls were all intact and no damage observed, and (B) *Aspergillus fumigatus*, showing severe destruction of the villi (H & E 100x)

Discussion:

Animal models are invaluable in enhancing our understanding of pathogen virulence, disease pathogenesis, and the feasibility of various therapies (31). Previous studies have shown that approximately 50.0% of birds can serve as reservoirs for fungi, which can be pathogenic to both humans and birds (32). The present study aimed to assess the health risks associated with the oral exposure of day-old chicks to spores from common filamentous fungi; *C. verruculosa, A. tubingensis, C. bertholletiae, F. oxysporum* and *A. fumigatus*. The results revealed varied clinical signs, symptoms, morphological changes, and mortality rates among the exposed birds.

Pathogenicity of *Curvularia verruculosa*

Historically, *Curvularia* species were considered non-pathogenic, however, recent reports suggest their increasing involvement in fungal diseases (33,34). In this study, chicks exposed to *C. verruculosa* exhibited symptoms such as loss of balance, frayed feathers, and reduced reflexes within five days. Additionally, necropsy findings showed intestinal obstruction and sloughing with oral ingestion of *C. verruculosa* (Plate 2B) consistent with Johnston et al., (35), who reported that fungi can disintegrate and obstruct the intestines. The high mortality rate (80.0%) observed in the infected bird supports previous reports that *C. verruculosa* can cause significant morbidity in birds (36).

The immune response in birds plays a crucial role in fighting infections, as their elevated body temperature (>40°C) and specific white blood cells help defend against pathogens (37). However as noted by Lowenthal et al., (38), the immature immune systems of day-old chicks may have contributed to their susceptibility to *C. verruculosa* infection. While previous studies suggest immunity in birds is linked to their innate and acquired systems (39), the present findings highlight the vulnerability of chicks, particularly through the observed morphological changes and mortality.

Pathogenicity of *Aspergillus fumigatus* and *Aspergillus tubingensis*:

Aspergillus fumigatus is the most commonly isolated fungal pathogen from both wild and domesticated birds (40), with known pathogenicity in respiratory and gastrointestinal systems. In this study, birds exposed to *A. fumigatus* and *A. tubingensis* displayed signs such as lethargy, diarrhoea, and dehydration, with mortality rate of 60.0% (3/5). This aligns with previous findings that *A. fumigatus* can cause significant morbidity and mortality in young birds (41). While fungal infections of the gastrointestinal tract are generally rare (42), the results suggest that these fungi can indeed induce gastrointestinal pathology, as evidenced by the clinical signs and histopathological findings of severe mucosal damage produced by *A. tubingensis* (Plate 5A) and *A. fumigatus* (Plate 8B).

Although study on mycotoxin was not carried out in our study, research studies have demonstrated that aflatoxin exposure is linked to various grades of gastrointestinal disorders and reproductive toxicity (6,42). The observed pathologies may therefore be associated with the presence of mycotoxin and further studies on this need to be carried out.

Pathogenicity of *Fusarium oxysporum* and *Cunninghamella bertholletiae:*

Infection with *F. oxysporum* led to notable pathologies, including rectal swelling (Plate 4A) and severe intestinal ulceration (Plates 7B and 8A). Despite these pathologies, the birds exposed to *F. oxysporum* had the highest survival rate of 80.0% (4/5) among the fungal isolates tested, indicating that while the infection was severe, it was less lethal compared to *C. verruculosa* and *C. bertholletiae.*

Necropsy findings of predeveloped intestinal walls suggest that the severity of the infection may have varied across individual birds. Similarly, *C. bertholletiae* caused extensive damage, as evidenced by severe intestinal haemorrhage and vessel invasion observed in the infected birds (Plate 5B). The high mortality rate (80.0%) agrees with the study by De Lucca (43), who reported that mucormycosis, often caused by *Cunninghamella* spp, carries a high mortality risk, particularly in cases of gastrointestinal involvement.

Mortality rate of day-old chicks:

The one-way ANOVA revealed a statistically significant difference in mortality rates between the different groups ($p<0.001$). Post-hoc comparisons indicated that the control group had a significantly lower mortality rate compared to all other groups. *C. verruculosa* and *C. bertholletiae* exhibited significantly higher mortality rates compared to *F. oxysporum. A. fumigatus* and *A. tubingensis* had mortality rates that were not significantly different from each other, but were significantly higher than *F. oxysporum*. These findings suggest that exposure to different fungal isolates resulted in varying levels of mortality in day-old chicks, with *C. verruculosa* and *Cunninghamella* spp. being the most pathogenic.

Role of the bursa of Fabricius:

Discolouration and enlargement of the bursa of Fabricius in birds exposed to *A. tubingensis, C. bertholletiae,* and *F. oxysporum* (Plates 3A, 3B, 4A and 4B) indicate its involvement in the immune response to these fungi. The bursa of Fabricius is crucial for the development of B-lymphocytes, essential for antibody production (44,45). Discolouration and enlargement of this organ suggest impaired immune function in the infected birds, which could have exacerbated the effects of the fungal infections. Contrary to these findings, El-Sharkawy et al., (11) reported no significant impact on the bursa of Fabricius in their study of fungal infections in broilers, suggesting that the roles of this organ may vary depending on the developmental stage of the bird or the fungal species involved.

Histopathological findings:

Histological analysis revealed significant pathological changes in the intestinal tissues of birds exposed to the fungal isolates. These ranged from mucosal distortion with *A. tubingensis* exposure (Plate 5A) to severe destruction of the villi with *A. fumigatus* (Plate 8B) and intestinal haemorrhage with *C. bertholletiae* (Plate 5B). These results are in contrast to earlier reports suggesting that the high body temperature of birds (>42°C) and their macrophage cells provide effective defence against fungal invasion (35,37). Findings of the study suggest that the fungi tested were able to bypass these defence mechanisms, leading to severe gastrointestinal pathology.

Conclusion:

This study highlights the pathogenic potential of several fungal species on day-old chicks, with *C. verruculosa* and *C. bertholletiae* posing the highest mortality risks. The observed discoloration and enlargement of the bursa of Fabricius suggest compromised immune function, exacerbating the severity of these infections. The high mortality rates and significant intestinal damage underscore the need for improved management of fungal contamination in poultry environments. These findings not only reveal the potential for substantial economic losses in poultry farming but also suggest possible zoonotic risks.

Further research is necessary to understand the role of fungal toxins in the observed pathology and to explore potential mitigation strategies, including vaccines, antifungal treatments, and biosecurity measures. This knowledge is essential for developing targeted interventions to safeguard poultry health and productivity.

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Contributions of author:

The author conceived and conducted the study, and wrote the manuscript.

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Conflict of interest:

The author declares no conflicts of interest regarding the publication of this paper

References:

1. Mahalingam, S.S., Jayaraman, S., and Pandiyan, P. Fungal Colonization and Infections – Interactions with Other Human Diseases. Pathogens. 2022; 11 (2): 212. https://doi.org/10.3390/pathogens11020212
2. Aruwa, C. E., Pillay, C., Nyaga, M. M., and Sabiu, S. Poultry gut health-microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. J Anim Sci Biotechnol. 2021; 12 (1): 119. doi: 10.1186/s40104-021-00640-9.
3. Chao, A., and Vazquez, J.A. Fungal infections of the gastrointestinal tract. Gastroenterol Clin North Am. 2021; 50 (2): 243-260. doi: 10.1016/j.gtc.2021.02.009
4. Carpouron, J. E., de Hoog, S., Gentekaki, E., and Hyde, K.D. Emerging Animal-Associated Fungal Diseases. J Fungi (Basel). 2022; 8 (6): 611. doi: 10.3390/jof8060611.
5. Radwan, I. A., Abed, A. H., and Abdallah, A. S. Prevalence of fungal pathogens in broiler chickens and their environment. J Vet Med Res. 2018; 25 (2): 174-181. doi:10.21608/jvmr.2017.43316
6. Mba, A. N., Ekwealor, C. C., Ogbukagu, C. M., Ekwealor, I. A., and Chukwuezi, F. O. Occurrence and Distribution of Fungal Isolates from Sputum, Ears and Nose Samples of Poultry Farm Workers in Anambra State, Nigeria. Am J Infect Dis Microbiol. 2020;8 (4):139-145. doi: 10.12691/ajidm-8-4-4
7. Gomes, B., Pena, P., Cervantes, R., Dias, M., and Viegas, C. Microbial Contamination of Bedding Material: One Health in Poultry Production. Int J Environ Res Publ Health. 2022; 19 (24): 16508. doi: 10.3390/ijerph192416508.
8. Ekwealor, C. C., Mba, A. N., Anakwenze, V. N., Anaukwu, C. G., and Ekwealor, A. I. Efficacy of methanol extracts of *Lawsonia inermis* L. and *Cymbopogon citratus* Stapf. against fungal isolates from poultry farms in Anambra State, Nigeria. J Pharm Biores. 2024; 21 (3): 135-143 doi:10.4314/jpb.v21i3.5
9. Crameri, R., Weichel, M., Flückiger, S., Glaser, A. G., and Rhyner, C. Fungal allergies: yet unsolved problem. Chem Immunol Allergy. 2006; 91: 121–133. doi: 10.1159/000090276.
10. Hernandez, A. J. C. Poultry and avian diseases. Encyc Agric Food Syst. 2014; 504-520 doi: 10.1016/B978-0-444-52512-3.00183-2
11. El-Sharkawy, H., El-Salam, A. M. A., and Tahoun, A. Pathology and epidemiology of fungal infections in layer chicken flocks. Adv Gut Microb. Res. 2023; 1: 1-13. doi:10.1155/2023/9956074
12. Ghetas, A. M., Sedeek, D.M., Fedawy, H. S., et al. Detection of intestinal fungi in chickens naturally infected with infectious bursal disease. Adv Anim Vet Sci. 2022; 10 (3): 514-520. doi.org/10.17582/journal.aavs/2022/10.3.514.520
13. Celi, P., Cowieson, A. J., Fru-Nji, F., Steinert, R. E., Kluenter, A-M., and Verlhac, V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. Anim Feed Sci Technol. 2017; 234: 88-100, https://doi.org/10.1016/j.anifeedsci.2017.09.012.
14. Li, J., Chen, D., Yu, B., et al. Fungi in Gastrointestinal Tracts of Human and Mice: from Community to Functions. Microb Ecol. 2018; 75 (4): 821-829. doi: 10.1007/s00248-017-1105-9.
15. Mgbeahuruike, A. C., 1agina, O. A., 1anyanwu, M. U., et al. Microbial Contamination of Poultry Feed and the Effects on Birds' Performance. Anim Res Int. 2023; 20 (1): 4834 – 4861
16. Dulisz, B., Dynowska, M., and Nowakowski, J. J. Body condition and colonization by fungi of House Sparrows Passer domesticus in the urban and rural environment. Euro Zoolog J. 2021; 88 (1): 152-164. doi:10.1080/24750263.2020.1857447
17. Casadevall, A., and Pirofski, L. Host-Pathogen Interactions: Basic Concepts of Microbial Commensalism, Colonization, Infection, and Disease. Infect Immun. 2000; 68: 12. https://doi.org/10.1128/iai.68.12.6511-6518.2000
18. Lamps, L.W., Lai, K. K., and Milner, D. A. Jr. Fungal

infections of the gastrointestinal tract in the immunocompromised host: an update. Adv Anat Pathol. 2014; 21 (4):217-227.
19. Mba, A. N., Ekwealor, C. C., and Ekwealor, I. A. Fungal Contaminants of Feed, Litter and Faecal Dropping Samples of Poultry Birds and their Occurrence and Distribution in Selected Poultry Farms in Anambra State, Nigeria. Nig J Microbiol. 2024; 38 (1): 6859-6870
20. De Hoog, G. S., Guarro, J., Gene, J. and Figueras, M. J. Atlas of Clinical Fungi. 2000; 2 (1). Centraal bureau voor Schimmel cultures, Utrecht, The Netherlands.
21. Diba, K., Kordbacheh, P., Mirhendi, S. H., Rezaie, S., and Mahmoudi, M. Identification of *Aspergillus* species using morphological characteristics. Pak J Med Sci. 2007; 23 (6): 867- 872.
22. Nadia, E. A., Ibtissem, B. S., Mohamed, B. K., Mahmoud, M., and Naima, B. Isolation and identification of fungal communities in organic and conventional soils. Int J Curr Microbiol Appl Sci. (2017); 6 (4): 1111-1123 doi:10.20546/ijcmas.2017.604.138
23. Chhabra, D., and Dhakad, N. K. Study on Pathogenicity of the *Aspergillus* species in experimentally immunosuppressed mice. Vet World. 2008; 1(3): 69-70
24. Dhama, K., Chakraborty, S., Verma, K. A., et al. Fungal/mycotic diseases of poultry-diagnosis, treatment and control: A Review. Pak J Bio Sci. 2013; 16: 1626-1640 doi: 10.3923/pjbs.2013.1626.1640
25. Beernaert, L. A., Pasmans, F., Van Waeyenberghe, L., Haesebrouck, F., and Martel, A. *Aspergillus* infections in birds: a review. Avian Pathol. 2010; 39 (5): 325-331 doi: 10.1080/03079457.2010.506210.
26. Oladele, O. O., Ameji, N. O., Odey, M., et al. Management of acute aspergillosis in newly hatched chicks: a case report. Nig Vet J. 2022; 43 (4): 64-73
27. Sharma, J. M., et al. Fungal Diseases of Poultry. In: Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., and Nair, V. (Eds.). Diseases of Poultry, 13th edn. Ames, IA: Wiley-Blackwell. 2013: 981-996.
28. McLelland, D. A., and Moffett, P. J. Avian Aspergillosis. In B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, & Y. M. Saif (Eds.), Diseases of Poultry. 10th edn. Ames, IA: Iowa State University Press 1994: 91- 802.
29. Morris A. J., Byrne, T. C, Madden, J, F. and Reller, L. B. Duration of incubation of fungal cultures. J Clin Microbiol. 1996; 34 (6): 1583-1585 doi:10.1128/jcm.34.6.1583-1585.1996.
30. Luna, L. G. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. New York, NY, USA: McGraw-Hill; 1968: 111-112.
31. Clemons, K. V., and Stevens, D. A. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med Mycol. 2005; 43 (Suppl 1): S101-110. doi:10.1080/13693780500051919.
32. Mirhosseini, Z., and Khosravi, A. Fungal Pathogens: Emerging Threats to Birds and Human Health, Assessment of the Relative Frequency of Pathogenic Fungi in Ornamental Bird Feces. J Poultry Sci Avian Dis. 2023; 1 (4): 20-24. http://dx.doi.org/10.61838/kman.jpsad.1.4.4
33. Carter, E., and Boudreaux, C. Fatal Cerebral Phaeohyphomycosis Due to *Curvularia lunata* in an Immunocompetent Patient. J Clin Microbiol. 2004; 42: https://doi.org/10.1128/jcm.42.11.5419-5423.2004
34. Krizsán, K., Papp, T., Manikandan, P., et al. Clinical Importance of the Genus *Curvularia.* In: Medical Mycology: Current Trends and Future Prospects. In Medical Mycology. 2016; 148-204
35. Johnston, S. A., Voelz, K., and Robin, C. M. *Cryptococcus neoformans* Thermotolerance to Avian Body Temperature Is Sufficient for Extracellular Growth but Not Intracellular Survival In Macrophages. Sci Rep. 2016; 6: 20977. doi: 10.1038/srep20977
36. Severo, C. B., Oliveira, F. M., Pilar, E. F. S., and Severo, L. C. Phaeohyphomycosis: a clinical-epidemiological and diagnostic study of eighteen cases in Rio Grande do Sul, Brazil. Mem Inst Oswaldo Cruz. 2012; 107 (7): 854-858. doi:10.1590/s0074-02762012000700003
37. Lowenthal, J. W., Connick, T. E., McWaters, P. G., and York, J. J. Development of T cell immune responsiveness in the chicken. Immun Cell Biol. 1994; 72 (2): 115-122. doi: 10.1038/icb.1994.18
38. Pavlidis, H. Avian Immunity 101: The basics. Poultry World. 2019. https://www.poultryworld.net/health-nutrition/avian-immunity-101-the-basics/
39. Arné, P., Risco-Castillo, V., Jouvion, G., and Guillot, J. Aspergillosis in wild birds. J Fungi (Basel). 2021; 7 (3): 241. doi: 10.3390/jof7030241
40. Ulloa-Avellán, O., Calderón-Hernández, A., Rubí Chacón, R., and Bernardo Vargas Leitón. *Aspergillus* spp Isolated from Lungs of Poultry (*Gallus gallus*) at the Mycology Laboratory, School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica between 2008 and 2021 and Associated Factors. J Fungi (Basel). 2023; 9 (1): 58. doi: 10.3390/jof9010058
41. Maragkoudakis, E., Realdi., G., and Dore, M. P. Fungal infections of the gastrointestinal tract. Recenti Prog Med. 2005; 96 (6): 311–317.
42. World Health Organization (WHO). Food Safety Digest: Aflatoxins. Department of Food Safety and Zoonoses, World Health Organization, Geneva. 2018
43. De Lucca, J. A. Harmful fungi in both agriculture and medicine. Rev Iberoam Micol. 2007; 24: 3-13
44. Gitlin, D. A., and Nussenzweig, M. C. Immunology: fifty years of B lymphocytes. Nature. 2015; 517 (7533):139–141 doi: 10.1038/517139a
45. Nera, K., Kyläniemi, M. K., and Lassila, O. Bursa of Fabricius. In: eLS. John Wiley and Sons Ltd, Chichester. doi: 10.1002/9780470015902.a0000506.pub4

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Original Article **Open Access**

Antimicrobial susceptibility pattern of *Salmonella* isolates from clinical, environmental and food sources in Lagos, Nigeria

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Abstract:

Background: Salmonellosis persists in Lagos, Nigeria, despite interventions. This study investigated antimicrobial resistance patterns of *Salmonella* species from clinical, food, and environmental samples in Lagos.
Methodology: From June to December 2018, 276 samples (86 blood, 80 stool, 40 hospital effluents, and 70 food) were collected for microbiological analysis. Clinical samples (blood and stool) were collected from patients in General Hospital Igando and Lagos State University Teaching Hospital Ikeja. Food samples were collected from food vendors in the urban periphery of Lagos State, and hospital effluents were collected from Gbagada General Hospital and Ojo Health Centre. The samples were processed using standard microbiological procedures. Antimicrobial susceptibility of isolated bacteria was done using the Kirby-Bauer disk diffusion method.
Results: Of the 276 samples, an overall prevalence of *Salmonella* spp from all sources was 9.1% (25/276), with 7.0% from blood, 17.5% from stool, 10.0% from hospital effluents, and 1.4% from food samples. *Salmonella* strains showed multidrug resistance (MDR) to at least four of nine antibiotics, with 98.0% resistance to ampicillin, nitrofurantoin and augmentin, and 99.0% sensitivity to imipenem. Five unique resistance patterns (A1-A5) were identified, with pattern A1 (AMP AUG GEN OFX CXM CTZ CIP NIT) observed in 72.0% of the *Salmonella* isolates, followed by A5 (AMP AUG GEN CXM CTZ NIT) in 8.0% of the isolates from two sources (stool and hospital effluent). The remaining patterns (A2, A3, and A4) were exhibited by *Salmonella* isolates from a single source.
Conclusion: The findings of this study suggest potential multi-source transmission dynamics of the investigated *Salmonella* species. It highlights the circulation of MDR *Salmonella* species from food and environmental sources with possible transmission to humans, emphasizing the need for continuous surveillance and control strategies. The persistence of *Salmonella* species and antimicrobial resistance in Lagos underscores the importance of targeted public health interventions to mitigate this public health threat.

Keywords: *Salmonella* species, antibiotic resistance, community acquisition, transmission dynamics.

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Profil de sensibilité aux antimicrobiens des isolats de *Salmonella* provenant de sources cliniques, environnementales et alimentaires à Lagos, au Nigéria

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Résumé:

Contexte: La salmonellose persiste à Lagos, au Nigéria, malgré les interventions. Cette étude a examiné les profils de résistance aux antimicrobiens des espèces de *Salmonella* provenant d'échantillons cliniques, alimentaires et environnementaux à Lagos.

Méthodologie: De juin à décembre 2018, 276 échantillons (86 de sang, 80 de selles, 40 d'effluents hospitaliers et 70 d'aliments) ont été collectés pour analyse microbiologique. Des échantillons cliniques (sang et selles) ont été prélevés auprès de patients de l'hôpital général d'Igando et de l'hôpital universitaire d'État de Lagos d'Ikeja. Des échantillons d'aliments ont été prélevés auprès de vendeurs de nourriture dans la périphérie urbaine de l'État de Lagos, et des effluents hospitaliers ont été prélevés à l'hôpital général de Gbagada et au centre de santé d'Ojo. Les échantillons ont été traités à l'aide de procédures microbiologiques standard. La sensibilité aux antimicrobiens des bactéries isolées a été réalisée à l'aide de la méthode de diffusion sur disque de Kirby-Bauer.
Résultats: Sur les 276 échantillons, la prévalence globale de Salmonella spp de toutes les sources était de 9,1% (25/276), avec 7,0% provenant du sang, 17,5% des selles, 10,0% des effluents hospitaliers et 1,4% des échantillons alimentaires. Français Les souches de *Salmonella* ont montré une résistance multidrogue (MDR) à au moins quatre des neuf antibiotiques, avec une résistance de 98,0% à l'ampicilline, à la nitrofurantoïne et à l'augmentin, et une sensibilité de 99,0% à l'imipénème. Cinq profils de résistance uniques (A1-A5) ont été identifiés, le profil A1 (AMP AUG GEN OFX CXM CTZ CIP NIT) étant observé dans 72,0% des isolats de *Salmonella*, suivi de A5 (AMP AUG GEN CXM CTZ NIT) dans 8,0% des isolats provenant de deux sources (selles et effluents hospitaliers). Les profils restants (A2, A3 et A4) ont été présentés par des isolats de *Salmonella* provenant d'une seule source.
Conclusion: Les résultats de cette étude suggèrent une dynamique potentielle de transmission multisource des espèces de *Salmonella* étudiées. Elle met en évidence la circulation des espèces de *Salmonella* MDR à partir de sources alimentaires et environnementales avec une possible transmission à l'homme, soulignant la nécessité de stratégies de surveillance et de contrôle continues. La persistance des espèces de Salmonella et de la résistance aux antimicrobiens à Lagos souligne l'importance d'interventions de santé publique ciblées pour atténuer cette menace pour la santé publique.

Mots-clés: espèces de *Salmonella*, résistance aux antibiotiques, acquisition communautaire, dynamique de transmission.

Introduction:

Salmonella enterica-associated infections (salmonellosis) can range in severity in humans from self-limiting gastroenteritis such as non-typhoidal *Salmonella* (NTS) to more severe typhoid, paratyphoid fever and invasive NTS (iNTS). Salmonellosis has a large macroeconomic impact on the world and causes significant illness and mortality in humans and animals (1). However, in Africa, NTS infections appear to be endemic, and iNTS is one of the major causes of bacteraemia, mostly in children, with 4,100 deaths per year (2). This condition is more prevalent in areas with high rates of malaria, malnutrition, and HIV (2). In developed countries, iNTS disease primarily affects young infants, the elderly, and immunocompromised individuals (3).

Invasive NTS disease is caused mainly by *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Salmonella enterica* serovar Enteritidis (*S*. Enteritidis) (4,5). While *S.* Typh*i* and *S.* Paratyphi are exclusively transmitted through human reservoirs, NTS gastroenteritis, in contrast, is commonly acquired from animal sources (6) and occurs through multiple routes, including consumption of contaminated animal products such as eggs, poultry and undercooked meats, contaminated produce, contact with animals or their environment, vectors such as houseflies, and contaminated water (1). The relative role of animal reservoirs and human-to-human transmission of strains causing iNTS disease is unclear (7, 8). Definitive diagnosis of iNTS disease relies on blood or bone marrow cultures, although these methods often exhibit low sensitivity (9). Furthermore, relying solely on clinical symptoms for diagnosis is unreliable due to the absence of any pathognomonic features (4,5).

Enteric fever, comprising both typhoid and paratyphoid fever, is a systemic infection caused by the human-specific pathogens *S.* Typhi and *S.* Paratyphi serotypes A, B, and C (10). Enteric fever remains a substantial contributor to global morbidity and mortality. As of 2010, an estimated 26.9 million cases and approximately 200,000 deaths were directly or indirectly attributed to typhoid fever annually (10). Low-and-middle-income countries (LMICs) are excessively affected by typhoid and paratyphoid fever, contributing to nearly half of the global morbidity and mortality, with an estimated 11.9 million cases and 129,000 deaths annually (6, 11).

The Global Burden of Disease (GBD) study, conducted by the Institute for Health Metrics and Evaluation, provides additional evidence of the significant global impact of typhoid and paratyphoid fevers. Their 2016 model estimated approximately 15.5million cases and 154,000 deaths attributed to these infections (12). However, the true incidence of typhoid fever and invasive NTS are difficult to evaluate in Nigeria because of the lack of a proper coordinated epidemiological surveillance system (6). Nevertheless, prevalence information on typhoid fever has been documented by several researchers in some States in Nigeria, ranging from 0.071% in Oyo (13), 47.1% in Osun (14), 7-18.6% in Lagos (6), 3.9-10.4% in Kano (6), and 0.8-2.4% in Abuja (6), over 23 years period among others.

Salmonellosis can be treated with app-

ropriate antibiotics, however, resistance to the first- and second-line antibiotics is a growing public health concern (6). Effective preventive vaccines for typhoid exist, and new vaccines are being developed while none exist for NTS, but corresponding investments in portable drinking water and sanitation infrastructure, food safety, hygiene practice by food handlers, environmental sanitation, and improved living conditions are insufficient (6,15). In Nigeria, both typhoid fever and NTS infections continue to pose significant health challenges due to factors such as rapid urbanization, insufficient access to clean water, the movement of migrant laborers across regions, and inadequate sanitation facilities (6). Other factors are overburdened healthcare delivery systems and overuse of antibiotics that contribute to the development and spread of antibiotic-resistant *S.* Typhi and NTS-causing serotypes such as *S.* Typhimurium, *S.* Enteritidis, and *S.* Cholerasuis (16,17).

Emergence and re-emergence of multidrug-resistance (MDR) strains in *Salmonella enterica* serotypes from clinical and environmental samples in different parts of the world have however been documented in the literature (18). This is coupled with the fact that our environment has recently been grossly polluted with huge refuse dumps (19). This study therefore investigated the occurrence and antimicrobial resistance patterns of *Salmonella* species from environmental samples and compared with isolates from fresh cases of *Salmonella*-associated diseases, which became necessary, in order to determine its transmissibility from the environment to humans through the faeco-oral routes in Lagos, Nigeria.

Materials and method:

Study area and sample collection:

The study was conducted in Lagos State, Nigeria. Clinical samples (blood and stool) were collected from patients at Alimosho General Hospital in Igando and Lagos State University Teaching Hospital in Ikeja. Additionally, food samples were collected from 35 vendors in the urban periphery of Lagos State, and hospital effluents were collected from Gbagada General Hospital and Ojo Health Centre.

Ethical consideration:

The Human Research and Ethics Committee of the Lagos State University Teaching Hospital with reference number LREC/06/10/961 approved the study, which was conducted in compliance with the 1964 Helsinki Declaration and any subsequent revisions or ethical standards of the same nature. Written informed consent of each particiapant or their legal guardian was obtained before samples were collected.

Study participants and clinical samples:

The participants for the clinical sampling were patients from the two randomly selected hospitals in Lagos State who have some or all of the symptoms of enteric fever such as fever, diarrhoea, abdominal discomfort, nausea, and vomiting. A total of 166 participants were recruited and venous blood samples were aseptically collected from 86 participants and stool samples from 80 participants.

Food samples:

A total of 35 eateries and food vendors from urban and outskirts areas of Lagos were selected for food sampling, collecting 2 samples each (70 samples in total) within 2 weeks. Risk factors such as stagnant water, improper utensil exposure, protective clothing, and debris were collected from the vendors.

Hospital effluent discharge:

A total of 40 hospital effluent samples, with 30 samples from Gbagada General Hospital (GGH) and 10 samples from Ojo Health Centre (OHC), were collected between June and December 2018.

Isolation and identification of *Salmonella* isolates:

Blood samples were cultured by conventional method with inoculation of sample into 50ml of thioglycollate broth (Oxoid, UK) and incubated at 37°C for 7 days, and turbidity was observed daily. After incubation, turbid tubes were subcultured on MacConkey agar (Oxoid, UK) plates. Stool samples were inoculated into Selenite-F broth (Oxoid, UK) and incubated for 24 hours at 37ºC.

Food samples were blended in a sterile blender jar with 9 ml of peptone water, and serial dilutions were carried out using the pour plate technique. Molten MacConkey agar (Oxoid, UK) was dispensed over the samples, mixed by rotating plates on workbenches, and inverted for 24-48 hours. Sterile universal bottles containing effluents were flamed before transferring 1 ml into 9 ml of peptone water for pre-enrichment. Turbid bottles were sub-cultured on MacConkey agar (Oxoid, UK) and eosin methylene blue agar (Oxoid, UK) and incubated at 37°C for 24 hours.

Colonies on the culture plates were further subcultured onto *Salmonella-Shigella* agar (SSA) (Oxoid, UK) and incubated overnight at 37°C. Discrete fresh colonies were Gram-stained, and biochemical tests were used for identification in accordance with Cowan and Steel (20).

Biochemical identification of *Salmonella* spp:

The Microbact™ (Oxoid, UK) identification kits were used following the manufacturer's instructions to phenotypically confirm the species identity of the isolates. The isolated bacteria were processed, subcultured onto nutrient agar slants, and stored at -4°C until further use.

Serotyping *Salmonella* isolates:

Two *Salmonella* antisera kits™ (SSI Diagnostica A/S, Denmark) were used for serotyping of the isolates; *Salmonella* O–serogroup kit was used for the identification of *S.* Typhi and *S.* Paratyphi, and *Salmonella* sero-Quick ID kit for *S.* Enteritidis and *S.* Typhimurium, following the manufacturer's instructions.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility was performed on all phenotypically confirmed isolates by the disc diffusion (Kirby-Bauer) method on Mueller-Hinton (MH) agar (Oxoid, UK) according to the guidelines of the Clinical and Laboratory Standards Institute (21). Briefly, positive isolates were tested against 9 antimicrobial agents. Test isolates and control strains were emulsified in sterile saline using a sterile wire-loop, and their turbidity was compared to a 0.5 McFarland standard solution. The bacterial suspension was swabbed onto MH agar, and excess moisture was removed before applying antimicrobial discs using a sterile forcep. The antibiotic discs (Rapid Labs, UK) used include amoxicillin/clavulanate (AUG 30μg), nitrofurantoin (NIT, 300μg), ceftazidime (CTZ, 30μg), ciprofloxacin (CIP, 5μg), ampicillin (AMP 10μg), ofloxacin (OFX 5μg), cefuroxime (CXM 30μg), gentamicin (GEN 10μg), and imipenem (IPM 10μg).

The inoculated plates were incubated at 37°C aerobically for 18-24 hours. The diameter of the zone of inhibition was measured (in mm) using a caliper and compared to a zone-interpretation chart of the CLSI to determine resistance or sensitivity in the isolates (21). A standard culture of *E. coli* ATCC 25922 was used as a quality control strain.

Statistical analysis:

Data analysis was performed using SPSS version 20.0. The Chi-square test was used to determine associations between qualitative variables, with *p*-value <0.05 considered statistically significant.

Results:

The overall prevalence of *Salmonella* spp isolation from all the samples was 9.1% (25/276), with 7.0% (6/86) from blood, 17.5% (14/80) from stool, 10.0% (4/40) from hospital effluents and 1.4% (1/70) from food sam ples (χ^2=14.13, p=0.0027) (Table 1). Specifically, prevalence of *Salmonella* isolation from stool samples (17.5%) was significantly higher (χ^2=6.35, p=0.012) than the overall prevalence (7.0%), while the prevalence was significantly lower from food samples (1.43%) (χ^2= 4.55, p=0.033), while there was no significant difference in the prevalence of *Salmonella* from blood (χ^2=0.41, p=0.51) and hospital effluent (χ^2=0.11, p=0.74) samples, with rates of 6.98% and 10.0% respectively (Table 1).

Comparative analysis shows that rate of *Salmonella* isolation from the stool samples was approximately 2.63 times more than from blood samples, 13.33 times more than from food samples, and 1.83 times more than from hospital effluent samples. Conversely, the rate of *Salmonella* isolation from hospital effluent samples was 7.14 times more than from food samples.

Table 1: Prevalence of *Salmonella* isolates from clinical and environmental sources in Lagos, Nigeria

Sample sources	The number and percentage of *Salmonella* Isolates			χ²	*p* value
	No. of samples	No. of isolates	Percentage		
Stool	86	6	6.98	6.35	0.012*
Blood	80	14	17.5	0.41	0.51
Hospital effluent	40	4	10	0.11	0.74
Food	70	1	1.43	4.55	0.033*
Total	**276**	**25**	**9.06**		

* = statistically significant at *p*<0.05

Table 2: Antibiotic resistance of *Salmonella* species isolated from different sources to selected antibiotics in Lagos, Nigeria

Source	Number of *Salmonella* isolates	Antibiotics (Percentage resistance)								
		AUG	GEN	CTZ	AMP	OFX	IPM	CXM	CIP	NIT
Blood	6	6 (100.0)	6 (100.0)	5 (83.3)	6 (100.0)	6 (100.0)	0	6 (100.0)	5 (83.3)	5 (83.3)
Stool	14	13 (92.9)	14 (100.0)	14 (100.0)	14 (100.0)	12 (86.0)	0	14 (100.0)	12 (86.0)	13 (92.9)
Hosptal effluent	4	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)	1 (25.0)	4 (100.0)	3 (75.0)	4 (100.0)
Food	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0	1 (100.0)	1 (100.0)	1 (100.0)
All sources	**25**	**24 (96.0)**	**25 (100.0)**	**25 (100.0)**	**25 (100.0)**	**22 (88.0)**	**1 (4.0)**	**25 (100.0)**	**21 (84.0)**	**23 (92.0)**

AUG: Amoxicillin/Clavulanate, NIT: Nitrofurantoin, AMP: Ampicillin, OFX: Ofloxacin, IPM: Imipenem, CXM: Cefuroxime, GEN: Gentamycin, CIP: Ciprofloxacin and CTZ: Ceftazidime.

All 25 (100.0%) *Salmonella* isolates were multidrug-resistant, with resistance to at least three antibiotic classes. For instance, all 6 (100.0%) *Salmonella* species from blood were resistant to cefuroxime, ofloxacin, gentamicin and amoxicillin-clavulanic acid while 5 (83.3%) of them were resistant to all the 9 antibiotics tested. However, all 14 (100.0%) isolates from stool samples were resistant to gentamicin, ceftazidime, ampicillin and cefuroxime, while 12 (86.0%) were resistant to eight antibiotics (Table 2).

Based on the speciation of 25 *Salmonella* isolates and their resistance phenotypes, the most frequently identified species was *S*. Typhimurium, accounting for 52.0% (13/ 25) of the isolates, followed by *S*. Typhi, which represented 32.0% (8/25) while the remaining 16.0% (4/25) were untypable *Salmonella* species. Remarkably, all 25 *Salmonella* species displayed resistance to most of the antibiotics tested, including third-generation cephalosporins.

The phenotypically typed *Salmonella* species exhibited five distinct resistance patterns, designated A1 to A5. The A1 resistance pattern (AMP AUG GEN OFX CXM CTZ CIP NIT) was the most prevalent (72.0%, 18/25) of the typed *Salmonella* species, which included 10 *S*. Typhimurium (8 from hospital effluents, 1 from food, and 1 from hospital effluent), 6 *S*. Typhi (4 from blood and 2 from stool), and 2 untypable *Salmonella* species. The A2 resistance pattern (AMP AUG GEN OFX CXM CTZ) was exhibited by 2 *S.* Typhi isolates from blood samples. The A3 resistance pattern (AMP AUG GEN CXM CTZ) was observed in 2 *S*. Typhimurium isolates from stool samples. The A5 resistance pattern (AMP AUG GEN CXM CTZ NIT) was displayed by 2 unserotyped *Salmonella* species (1 from stool and 1 from hospital effluent). The least common resistance pattern, A4 (AMP AUG GEN OFX IPM CXM CTZ CIP NIT), was exhibited by a single *S*. Typhimurium isolate from hospital effluent (Table 3).

Discussion:

Salmonellosis has been documented to be endemic in Nigeria (22). However, the transmission dynamics of the causative agent to this disease have been sparsely reported. Hence, this study investigated the antimicrobial resistance and the transmission dynamics of *Salmonella* species from diverse sources in Lagos, Nigeria. In this study, the prevalence of *Salmonella* isolates varied across different sample sources, reflecting diverse contamination levels and potential exposure risks. The aggregated prevalence rate of *Salmonella* across all sources was 9.06%, emphasizing its persistent circulation in Lagos. These results align with previous studies on environmental and clinical reservoirs of *Salmonella* (23), which reported MDR *Salmonella* isolates in similar contexts.

Specifically, stool samples yielded significantly higher rate of *Salmonella* isolation of 17.5% (χ^2=6.35, p=0.012) compared to the overall *Salmonella* isolation rate from all sources, indicating an association between stool samples and *Salmonella* isolation. This is not unexpected as *Salmonella* is majorly carried in the gastrointesinal tract, with significant presence in the stool. Conversely, a significantly lower *Salmonella* isolation rate of 1.43% (χ^2= 4.55, p=0.033) was recorded from food samples. Although this rate is low, it underscores the importance of foodborne transmission of *Salmonella* (24). Our findings highlight stool and food samples as critical surveillance points in managing *Salmonella* outbreaks (24).

The *Salmonella* isolation rate from blood samples of 6.98% was not significantly different from the overall *Salmonella* isolation rate from all sources (χ^2=0.41, p=0.51), and this is the same observation with hospital effluents, with rate of 10.0% (χ^2=0.11, p=0.74).

Table 3: Antibiotic resistance profile of *Salmonella* species isolated from different sources in Lagos, Nigeria

Serotypes	Sample source	Isolate number	Month of isolation	Antibiotype No	Antibiotic resistance profiles
Salmonella Typhi	Human	bld001	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		bld002	July 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		bld003	July 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		bld004	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		bld005	July 2018	A2	AMP AUG GEN OFX CXM CTZ
		bld006	July 2018	A2	AMP AUG GEN OFX CXM CTZ
		stl001	June, 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl002	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
Salmonella Typhimurium	Food	fds001	July 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
	Human	stl003	Dec 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl004	Dec 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl005	Dec 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl006	July 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl007	July 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl008	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl009	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl010	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl011	June 2018	A3	AMP AUG GEN CX MCTZ
		stl012	Dec 2018	A3	AMP AUG GEN CXM CTZ
	Hospital	hed001	June 2018	A4	AMP AUG GEN OFX IPM CXM CTZ CIP NIT
	Effluent	hed002	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
Untypable *Salmonella*	Human	stl013	Dec 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
		stl014	June 2018	A5	AMP AUG GEN CXM CTZ NIT
	Hospital	hed003	June 2018	A1	AMP AUG GEN OFX CXM CTZ CIP NIT
	Effluent	hed004	June 2018	A5	AMP AUG GEN CXM CTZ NIT

AUG: Amoxicillin/Clavulanate, NIT: Nitrofurantoin, AMP: Ampicillin, OFX: Ofloxacin, IPM: Imipenem, CXM: Cefuroxime, GEN: Gentamycin, CIP: Ciprofloxacin, CTZ: Ceftazidime

While both blood and hospital effluents contribute to the overall prevalence of *Salmonella*, our findings may indicate less consistent contamination or limitations in our study design, sample collection and processing (17,22). Overall, these findings underscore the need for targeted interventions in high-risk areas, particularly foodborne and stool-related contamination routes. Continuous surveillance, robust sampling methodologies, and public health strategies are crucial to addressing the endemic nature of *Salmonella* in Lagos, Nigeria.

The antibiotic resistance profiles in this study showed that all *Salmonella* species isolated from clinical samples were resistant to 4 of the 9 antibiotics evaluated, with all 6 *Salmonella* isolates from blood being resistant to cefuroxime, ofloxacin, gentamicin and amoxicillin-clavulanate, and 14 isolates from stool samples being resistant to gentamicin, ceftazidime, ampicillin, and cefuroxime. This result disagrees with the study of Anchau et al., (25), who reported that all the *Salmonella* isolates recovered in their study were sensitive to cefuroxime, ciprofloxacin and gentamicin. Contrarily, Gordana et al., (26) reported 2.5% resistance in *Salmonella* isolates to ciprofloxacin. In addition, 94.73% and 55.6% of *Salmonella* isolates were resistant to cefuroxime in the studies by Sivakumar et al., (27) and Ifeanyi et al., (28), respectively. The results of our study however, agrees with Beyene et al., (29), who reported 74.3% resistance in bacterial enteropathogens against gentamicin and cefuroxime. Nevertheless, a 100.0% resistance rate was observed against amoxicillin-clavulanate in our study, which was higher than the 75.0% reported by Nesa et al., (30).

Our observation of high antibiotic resistance in this study might be due to the extensive use in both human and animal medicine (17). It has been reported that extensive and indiscriminate use of antibiotics usually disrupts the normal flora of the intestine, resulting in the emergence of antibiotic-resistant enteric pathogens (including *Salmonella*) and prolonged faecal shedding of MDR bacteria into the environment (17,31). The progressive increase in antibiotic resistance among enteric pathogens is now a global issue, but particularly in developing countries. However, antibiotic susceptibility patterns vary regionally and geographically and have been reported to change rapidly over time (32). The source of isolation and variability of strains among the same serovar may influence antibiotic resistance patterns (33). In developing countries, poor or inadequate laboratory facilities, empirical prescription of broad-spectrum antimicrobials by clinicians based solely on symptomatology, among other factors, have aggravated the emergence of antibiotic-resistant bacterial patho-

gens (17,34,35).

Furthermore, this study observed that all the *Salmonella* species recovered from hospital effluents were resistant to 6 antibiotics, including gentamicin, amoxicillin-clavulanate, ceftazidime, ampicillin, cefuroxime and nitrofurantoin. This resistance pattern underscores the critical role of hospital effluents as reservoirs for MDR pathogens, which can disseminate into the environment and pose public health risks. Hospital effluents often contain high concentrations of antibiotics, creating 'selection pressure' that promotes survival and proliferation of resistant bacterial strains (36). *Salmonella* is particularly adept at acquiring resistance genes through horizontal gene transfer mechanisms, such as plasmids and transposons (37).

The resistance to multiple antibiotics, as observed in this study, limits therapeutic options and complicates the management of *Salmonella* infections, which can range from gastroenteritis to invasive diseases like septicaemia (18). The resistance to broad-spectrum antibiotics such as cefotaxime and amoxicillin-clavulanate is particularly alarming, as these are often used as first-line treatments for severe infections (37). This trend highlights the urgent need for stringent antibiotic stewardship programs in healthcare settings including minimizing release of antibiotics into hospital effluents (38). Additionally, advanced wastewater treatment technologies should be implemented to mitigate environmental dissemination of MDR pathogens (39). It should be noted that the observed resistance of *Salmonella* species to multiple antibiotics in hospital effluents is a stark reminder of the interconnectedness of human, environmental, and animal health (36). Addressing this issue requires a 'One Health' approach that integrates efforts across disciplines to effectively combat AMR (36).

Interestingly, the only *Salmonella* isolate (*S.* Typhimurium) recovered from the food sample (raw meat) was resistant to 8 of the 9 antibiotics tested including ampicillin, amoxicillin-clavulanate, gentamicin, ofloxacin, cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin. This result highlights the role of foodborne pathogens in spreading MDR bacteria, thereby complicating the treatment of infections, and increasing the risk of therapeutic failure (24). Similarly, Ejo et al., (40) and Wolde et al., (41) reported 5.5%, 40.0%, and 44.8% MDR *Salmonella* food-animal sources. Several other studies have reported similar findings (42-45) in livestock production. The use of antibiotics in livestock feeds as growth promoters and for prophylactic may promote in-farm selection of antimicrobial-resistant strains and markedly increase the human health risks associated with the consumption of contaminated food products (17,46).

Furthermore, David and Oluyege (47) reported high resistance against ampicillin in *Salmonella* isolates of 84.0% (239/284) and 94.1% (34/97) from food and food handlers, respectively. Other studies on similar findings include those of Afolabi and Oloyede (48), Karshima et al., (49), Smith et al., (50), and Olalekan et al., (51). Lack of proper education on good hygiene practices (51,52) and lack of formal knowledge of food preparation and hygiene are some of the factors promoting the spread of MDR bacteria in food (51,53,54). More importantly, this study underscores the need for stricter regulation of antibiotic use in livestock production, improved food hygiene practices, and alternative approaches to managing MDR pathogens.

Twenty-five (19.38%) of the *Salmonella* isolates, made up of three distinct serotypes, *S.* Typhi (n=8), *S.* Typhimurium (n= 13), and 4 untyeable *Salmonella*, exhibited 5 distinct antibiotypes (A1 to A5). Out of the 5 antibiotypes, A1 (AMP AUG GEN OFX CXM CTZ CIP NIT) was the most prevalent (72.0%) resistance pattern, cutting across all the *Salmonella* serotypes from each of the sample sources. This is an indication that food and hospital effluents may serve as vehicles for the transmission of drug-resistant *Salmonella* to humans. Our study, however, shared some similarities to the study of Akinyemi et al., (35,55), who reported common resistant patterns among clinical and environmental *Salmonella* isolates and that well and sachet water were likely sources and/or vehicles of transmission of MDR *Salmonella* species to humans. Similarly, Mechesso et al., (56) in 2017 also reported common resistant phenotypes among *Salmonella enterica* serovars from food-producing animals in South Korea. Our study indicates the need for comprehensive molecular typing and epidemiology of all the *Salmonella* isolates obtained in this study with other circulating strains in Nigeria to trace the epidemiological link through clonal relatedness and diversity.

Conclusion:

This study showed that drug-resistant *Salmonella* are now endemic in our environment. Molecular characterization of the isolates with emphasis on resistant strains is required to identify the mechanisms of resistance. Furthermore, our study showed that both typhoidal and non-typhoidal *Salmonella* are implicated in community-acquired bacteraemia in Nigeria, thus posing a threat to public health. Careful and rational use of antimicrobial agents, particularly carbapenem antibiotics

such as imipenem, in the treatment of bacteraemia is essential to preserve its efficacy. Epidemiological surveillance of typhoidal and non-typhoidal *Salmonella* across all the 36 States of the 6 geopolitical zones in Nigeria by the Nigeria Centre for Disease Control and Prevention (NCDC) is highly recommended.

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Contributions of authors:

ASO and SCO. collected blood samples and performed sample analysis. ASO wrote the first draft of the manuscript. AKO conceived and designed the study, and ASO and FCO contributed to sample analysis and manuscript writing. ARO, LAO and SWO contributed to the manuscript writing, and AKO edited the manuscript. All authors approved the final copy of the manuscript.

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References:

1. Smith, S., Seriki, A., and Ajayi, A. Typhoidal and non-typhoidal *Salmonella* infections in Africa. Eur J Clin Microbiol Infect Dis. 2016; 35: 1913-1922. https://doi.org/10.1007/s10096-016-2760-3
2. Majowicz, S. E., Musto, J., Scallan, E., et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis. 2010; 50 (6): 882-889. https://doi.org/10.1086/650733
3. Balasubramanian, R., Im, J., Lee, J-S., et al. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. Hum Vaccin Immunother. 2019; 15 (6): 1421-1426. https://doi.org/10.1080/21645515.2018.1504717
4. Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., and Gordon, M. A. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. Lancet. 2012; 379 (9835): 2489-2499. https://doi.org/10.1016/S0140-6736(11)61752-2
5. MacLennan, C. A., and Levine, M. M. Invasive nontyphoidal *Salmonella* disease in Africa: current status. Exp Rev Anti-infect Ther. 2013; 11 (5): 443-446. https://doi.org/10.1586/eri.13.27
6. Akinyemi, K. O., Oyefolu, A. O. B., Mutiu, W. B., et al. Typhoid fever: tracking the trend in Nigeria. Am J Trop Med Hyg. 2018; 99 (3): 41. https://doi.org/ 10.4269/ajtmh.18-0045
7. Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., and Hart, C. A. Characterisation of community acquired non-typhoidal *Salmonella* from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. BMC Microbiol. 2006; 6 (1): 1-10. https://doi.org/10.1186/1471-2180-6-101
8. Feasey, N. A., Archer, B. N., Heyderman, R. S., et al. Typhoid fever and invasive non-typhoid salmonellosis, Malawi and South Africa. Emerg Infect Dis. 2010; 16 (9): 1448. https://doi.org/10.3201/eid1609.100125
9. Ao, T. T., Feasey, N. A., Gordon, M. A., Keddy, K. H., Angulo, F. J., and Crump, J. A. Global burden of invasive nontyphoidal *Salmonella* disease, 2010. Emerg Infect Dis. 2015; 21 (6): 941. https://doi.org/ 10.3201/eid2106.140999
10. Buckle, G. C., Walker, C. L. F., and Black, R. E. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. J Glob Health. 2012; 2 (1): 010401. https://doi.org/10.7189/jogh.02.010401
11. Mogasale, V., Maskery, B., Ochiai, R. L., et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. Lancet Glob Health. 2014; 2 (10): e570-e580. https://doi.org/10.1016/S2214-109X(14)70301-8
12. Stanaway, J. D., Reiner, R. C., Blacker, B. F., et al. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Infect Dis. 2019; 19 (4): 369-381. https://doi.org/10.1016/S1473-3099(18)30685-6.
13. Fashae, K., Ogunsola, F., Aarestrup, F. M., and Hendriksen, R. S. Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. J Infect Dev Ctries. 2010; 4 (08): 484-494. https://doi.org/10.3855/jidc.909
14. Uyigue, P. O., and Anukam, K. Monitoring of enteric fever and diarrhea-causing bacteria in a rural setting in Nigeria. J Bacteriol Res. 2011; 3 (5): 88-91. doi:10.1093/ajcp/138.suppl1.278
15. Als, D., Radhakrishnan, A., Arora, P., et al. Global trends in typhoidal salmonellosis: a systematic review. Am J Trop Med Hyg. 2018; 99 (3 Suppl): doi: 10.10.4269/ajtmh.18-0034
16. Akinyemi, K., Smith, S., Oyefolu, A. B., and Coker, A. Multidrug resistance in *Salmonella enterica* serovar Typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. Publ Health. 2005; 119 (4): 321-327. https://doi.org/10.1016/j.puhe.2004.04.009
17. Akinyemi, K. O., and Ajoseh, S. O. Factors contributing to the emergence and spread of antibiotics resistance in *Salmonella* species. Current Topics in *Salmonella* and Salmonellosis. 2017: 97-114. doi: 10.5772/67701
18. Akinyemi, K. O., Ajoseh, S. O., and Fakorede, C. O. A systemic review of literatures on human *Salmonella* enterica serovars in Nigeria (1999-2018). J Infect Dev Ctries. 2021; 15 (09): 1222-1235.https://doi.org/10.3855/jidc.12186
19. Aluko, O. E. Environmental Degradation and the Lingering Threat of Refuse and Pollution in Lagos State. J Manag Sustainab. 2012; 2: 217. doi: 10.5539/jms.v2n1p2175
20. Uttley, A., Collins, C., Cowan and Steel's manual for the identification of medical bacteria. 3rd edn: Barrow, G. I., and Feltham, R. K. A. (Eds.) Cambridge University Press 1993: 331
21. Weinstein, M. P. Performance standards for antimicrobial susceptibility testing: Clinical and Laboratory Standards Institute; 2021.
22. Akinkunmi F. M., Ajoseh, S. O., Fakorede, C., et al. Prevalence of *Salmonella* bloodstream infection and Antimicrobial Resistance in Lagos, Nigeria. Infect Epidemiol Microbiol. 2023; 9 (1): 1-14. http://dx.doi.org/10.52547/iem.9.1.1

23. Jibril, A. H., Okeke, I. N., Dalsgaard, A., et al. Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. PLoS One. 2020;15(9):e0238190. https://doi.org/10.1371/journal.pone.0238190
24. Wang, Y., Ge, H., Wei, X., and Zhao, X. Research progress on antibiotic resistance of *Salmonella*. Food Quality and Safety. 2022; 6: fyac035. https://doi.org/10.1093/fqsafe/fyac035
25. Anchau, Z., Olonitola, O., and Ella, E. Prevalence and Antibiotic Susceptibility of *Salmonella* Species isolated from Patients attending Selected Hospitals in Zaria. Annals Exper Biol Biochem. 2016; 4: 1-6. doi:10.14196/sjmi.v5i3.2109
26. Gordana, M., Bogdanka, A., Dragica, T., Milena, L., and Brankca, D. Antibiotic susceptibility of *Salmonella* species A comparison of two surveys with 5 years interval. Journal of IMAB-Annual proceeding (Scientific papers). 2012; 18 (1): 243-68. doi: 10.5272/jimab.2012181.216
27. Sivakumar, T., Avinash Saravanavel, N., Prabhu, D., Shankar, T., and Vijayabaskar, P. Characterization of multidrug-resistant patterns of *Salmonella* sp. World J Med Sci. 2012;7(2):64-7. https://doi.org/10.4102/ajlm.v11i1.1606
28. Ifeanyi, C. I. C., Bassey, B. E., Ikeneche, N. F., and Al-Gallas, N. Molecular characterization and antibiotic resistance of *Salmonella* in children with acute gastroenteritis in Abuja, Nigeria.J Infect Dev Ctries. 2014 11;8(6):712-719. doi: 10.3855/jidc.4185.
29. Beyene, G., Nair, S., Asrat, D., Mengistu, Y., Engers, H., and Wain, J. Multidrug-resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. J Infect Dev Ctries. 2011; 5 (1): 23-33. doi: 10.3855/jidc.906.
30. Rahman, M., Rahman, A., Islam, M., and Alam, M. Detection of multi–drug resistant *Salmonella* from milk and meat in Bangladesh. Bangladesh J Veter Med. 2018;16 (1): 115-120. https://doi.org/10.3329/bjvm.v16i1.37388
31. Threlfall, E. J. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev. 2002; 2 6(2): 141-148. doi:10.1111/j.1574-6976.2002.tb00606.x
32. WHO. Improving infection prevention and control at the health facility: interim practical manual supporting implementation of the WHO guidelines on core components of infection prevention and control programmes. World Health Organization, 2018.
33. Shrivastava, S. R., Shrivastava, P. S., and Ramasamy, J. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. J Med Society. 2018; 32 (1): 76. doi: 10.4103/jms.jms_25_17
34. Okeke, I. N., Aboderin, O. A., Byarugaba, D. K., Ojo, K. K., and Opintan, J. A. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg Infect Dis. 2007 (11): 1640 - 1646. doi: 10.3201/eid1311.070674.
35. Akinyemi, K., Ajoseh, S., Iwalokun, B., et al. Antimicrobial resistance and plasmid profiles of *Salmonella* enterica serovars from different sources in Lagos, Nigeria. Health. 2018; 10 (6): 758-772. doi: 10.4236/health.2018.106058
36. Talukder, H., Roky, S. A., Debnath, K., Sharma, B., Ahmed, J., and Roy, S. Prevalence and antimicrobial resistance profile of *Salmonella* isolated from human, animal and environment samples in South Asia: a 10-year meta-analysis. J Epid Global Health. 2023; 13 (4): 637-652. doi: 10.1007/s44197-023-00160-x
37. Nazir, J., Manzoor, T., Saleem, A., et al. Combating *Salmonella*: a focus on antimicrobial resistance and the need for effective vaccination. BMC Infect. Dis. 2025;25 (1): 84. https://doi.org/10.1186/s12879-025-10478-5
38. Centre for disease Control and Prevention (CDC). Core elements of hospital antibiotic stewardship programs. Atlanta: CDC. 2024: 8-20. doi: 10.1093/cid/ciu542
39. Tolkou, A. K., and Kyzas, G. Z. Advanced Technologies of Water and Wastewater Treatment. MDPI; 2024: 270. https://doi.org/10.3390/environments11120270
40. Ejo, M., Garedew, L., Alebachew, Z., and Worku, W. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. BioMed Res Int. 2016; 2016. doi: 10.1155/2016/4290506.
41. Wolde, T., Abate, M., Sileshi, H., and Mekonnen, Y. Prevalence and antimicrobial susceptibility profile of *Salmonella* species from ready-to-eat foods from catering establishments in Jigjiga City, Ethiopia. Afr J Microbiol Res. 2016; 10 (37): 1555-1660. https://doi.org/10.5897/AJMR2016.7944
42. Stevens, A., Kaboré, Y., Perrier-Gros-Claude, J. D., et al. Prevalence and antibiotic-resistance of *Salmonella* isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal).Int J Food Microbiol. 2006 15; 110 (2): 178-186. doi: 10.1016/j.ijfoodmicro.2006.04.018.
43. Khaitsa. M. L, Kegode, R. B., and Doetkott, D. K. Occurrence of antimicrobial-resistant *Salmonella* species in raw and ready to eat turkey meat products from retail outlets in the midwestern United States. Foodborne Pathog Dis. 2007; 4(4): 517-525. doi: 10.1089/fpd.2007.0010
44. Elgroud, R., Zerdoumi, F., Benazzouz, M., et al. Characteristics of *Salmonella* contamination of broilers and slaughterhouses in the region of Constantine (Algeria). Zoonoses Publ Health. 2009; 56 (2): 84-93. doi: 10.1111/j.1863-2378.2008.01164.x
45. Fesseha, H., Aliye, S., Kifle, T., and Mathewos, M.. Isolation and multiple drug resistance patterns of *Salmonella* isolates from selected dairy farms in Hawassa town, Ethiopia. J Vet Sci Med. 2020; 8 (1): 7.
46. Zewdu, E., and Cornelius, P. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. Trop Ani health and prod. 2009; 41 (2): 241-249. doi: 10.1007/s11250-008-9181-y
47. David, O., and Oluyege, A. Antibiotic resistance and plasmid carriage among *Salmonella* Typhi isolated from food and hands of food handlers in a Nigerian University. Int J Curr Microbiol Appl Sci. 2015; 4 (3): 906-914.
48. Afolabi, O., and Oloyede, A. Irrigation water as possible source of food borne pathogens in raw vegetables. ASSET: An International Journal (Series B). 2012; 9 (2): 117-122.
49. Karshima, N., Pam, V., Bata, S., Dung. P., and Paman, N. Isolation of *Salmonella* species from milk and locally processed milk products traded for human consumption and associated risk factors in Kanam, Plateau State, Nigeria. J Ani Prod Adv. 2013; 3 (3): 69-74. doi: 10.5455/japa.20130330124355
50. Smith, S., Opere, B., Fowora, M., et al. Molecular characterization of *Salmonella* spp directly from snack and food commonly sold in Lagos, Nigeria. Southeast Asian J Trop Med Publ Health. 2012; 43 (3): 718-721.
51. Olalekan, A. W., Oluwaseun, F. A., and Oladele, H. A. W. Prevale nce and knowledge of *Salmonella* infections among food handlers: Implications for school health in Southwestern Nigeria. Sahel Med J. 2018; 21 (2): 99. https://doi.org/10.4103/smj.smj_27_16
52. Esan, C. Molecular studies of resistant *Staphylo-*

coccus aureus in Ekiti and Ondo States, Nigeria: Ph. D Thesis. University of Ado-Ekiti, Nigeria

53. Omemu, A. M., and Aderoju, S. Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. Food contr. 2008; 19 (4): 396-402. https://doi.org/10.1016/j.foodcont.2007.04.021
54. Adjrah, Y., Soncy, K., Anani, K., et al. Socio-economic profile of street food vendors and microbiological quality of ready-to-eat salads in Lomé. Int. Food Res. J. 2013; 20 (1): 65.
55. Akinyemi, K., Iwalokun, B., Foli, F., Oshodi, K., and Coker, A. Prevalence of multiple drug resistance and screening of enterotoxin (stn) gene in *Salmonella* enterica serovars from water sources in Lagos, Nigeria. Publ Health. 2011; 125 (2): 65-71. doi: 10.1016/j.puhe.2010.11.010.
56. Mechesso, A. F., Moon, D. C., Kim, S. J., et al. Nationwide surveillance on serotype distribution and antimicrobial resistance profiles of non-typhoidal *Salmonella* serovars isolated from food-producing animals in South Korea. Int J Food Microbiol. 2020, 16: 335: 108893. doi: 10.1016/j.ijfoodmicro.2020.108893.

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Original Article **Open Access**

Bacteriological quality of *Datura stramonium* cocktail ('gegemu') and antimicrobial susceptibility of isolated bacterial pathogens

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Abstract:

Background: *Datura stramonium,* commonly known as Jimson weed, is widely recognized in traditional medicine, and is a key ingredient in various herbal concoctions. Aside from the well-known herbal formulation, a cocktail made from *Datura stramonium*, known locally as 'gegemu', has become increasingly popular among students in our environment, raising concerns about its microbial quality. The fact that this cocktail is not commercially available raises the possibility of contamination by pathogenic microorganisms during preparation or storage, posing significant health risk. The objective of this study was to assess the microbial quality of this cocktail drink and determine the antimicrobial susceptibility of the bacteria isolates.
Methodology: Because this cocktail is not commercially available, a purposive sampling technique was adopted to collect 10 samples of the 'gegemu' cocktail from students in three selected tertiary institutions within Abeokuta metropolis, Nigeria. The samples were transported in ice packs to the biology laboratory of the Federal College of Education, Abeokuta, Nigeria for microbial analysis. The pH of the samples was determined using pH meter. Samples were cultured on plate count, nutrient, *Salmonella*-*Shigella*, Eosin Methylene Blue (EMB) and MacConkey agar plates using pour plate method for heterotrophic count. Isolated bacteria were identified by conventional methods as described in Bergey's Manual of Systematic Bacteriology. Antimicrobial susceptibility test (AST) was performed against 9 antibiotics using the Kirby-Bauer disk diffusion method.
Results: The mean pH of the samples was 3.705±0.005 with a range of 3.25±0.02 to 4.11±0.02. All samples were contaminated, with heterotrophic count ranging from mean value of 5.8±0.09x10^{5} for sample G4 to 7.9± 0.24x10^{6} CFU/ml for sample G8, which exceed the acceptable limit of bacteria content of drinks safe for consumption. A total of 30 bacteria isolates were recovered from the samples, and this includes *Staphylococcus aureus* (n=6), *Staphylococcus epidermidis* (n=2), *Bacillus cereus* (n=3), *Shigella flexneri* (n=3), *Salmonella* Typhi (n=4), *Pseudomonas aeruginosa* (n=3), *Escherichia coli* (n=4), *Klebsiella pneumoniae* (n=3) and *Vibrio cholerae* (n=2). The AST results indicated that *E. coli* was 99.4% sensitive to cefixime but only 10.7% to tetracycline, *S. flexneri* was 91.7% sensitive to gentamicin but only 21.6% to cefuroxime, *S.* Typhi was 100.0% sensitive to cefixime but only 19.1% to tetracycline, *V. cholerae* was 88.3% sensitive to cefixime but only 32.6% to ampicillin, *S. aureus* was 85.9% sensitive to cotrimoxazole but only 16.4% to tetracycline, and *S. epidermidis* was 92.4% sensitive to cotrimoxazole but only 23.7% to tetracycline. Other isolates such as *K. pneumoniae, P. aeruginosa* and *B. cereus* displayed varying levels of resistance to tetracycline and ampicillin.
Conclusion: Our study indicates that in spite of the acidity of the 'gegemu' cocktail, it contains pathogenic bacteria that exceeded the acceptable microbial thresholds, rendering it unsafe for consumption. Additionally, some of the isolated bacteria showed resistance to tetracycline and ampicillin, which are common antibiotics used in our environment. It is essential to enhance public health awareness to address and reduce these risks.

Keywords: bacteriological; heterotrophic count; 'gegemu'; cocktail; antimicrobial resistance

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Qualité bactériologique du cocktail *Datura stramonium* («Gegemu») et sensibilité aux antimicrobiens des agents pathogènes bactériens isolés

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Résumé:

Contexte: *Datura stramonium*, communément appelé Jimson weed, est largement reconnu en médecine traditionnelle et est un ingrédient clé dans diverses concoctions à base de plantes. Mis à part la formulation à base de plantes bien connue, un cocktail fabriqué à partir de *Datura stramonium* connu localement sous le nom de «Gegemu» est devenu de plus en plus populaire parmi les étudiants dans notre environnement, ce qui soulève des préoccupations concernant sa qualité microbienne. Le fait que ce cocktail ne soit pas disponible dans le commerce augmente la possibilité de contamination par des micro-organismes pathogènes pendant la préparation ou le stockage, posant un risque de santé important. L'objectif de cette étude était d'évaluer la qualité microbienne de ce cocktail et de déterminer la sensibilité antimicrobienne des isolats bactéries.
Méthodologie: Étant donné que ce cocktail n'est pas disponible dans le commerce, une technique d'échantillonnage ciblée a été adoptée pour collecter 10 échantillons du cocktail «Gegemu» des étudiants dans trois établissements d'enseignement supérieur sélectionnés au sein d'Abeokuta Metropolis, au Nigéria. Les échantillons ont été transportés dans des packs de glace au laboratoire de biologie du College d'Education Fédéral, Abeokuta, Nigéria pour l'analyse microbienne. Le pH des échantillons a été déterminé à l'aide du pH-mètre. Les échantillons ont été cultivés sur le nombre de plaques, les nutriments, les *Salmonelles-Shigella*, le bleu méthylène (EMB) et les plaques de gélose MacConkey en utilisant la méthode de la plaque pour le nombre hétérotrophique. Les bactéries isolées ont été identifiées par des méthodes conventionnelles telles que décrites dans le manuel de Bergey de la bactériologie systématique. Le test de sensibilité aux antimicrobiens (AST) a été effectué contre 9 antibiotiques en utilisant la méthode de diffusion du disque de Kirby-Bauer.
Résultats: Le pH moyen des échantillons était de 3,705±0,005 avec une plage de 3,25±0,02 à 4,11±0,02. Tous les échantillons ont été contaminés, avec un nombre hétérotrophique allant d'une valeur moyenne de $5,8\pm0,09\times10^5$ pour l'échantillon G4 à $7,9\pm0,24\times10^6$ CFU/ml pour l'échantillon G8, qui dépasse la limite acceptable de la teneur en bactéries des boissons sans danger pour la consommation. Au total, 30 isolats de bactéries ont été récupérés à partir des échantillons, ce qui comprend *Staphylococcus aureus* (n=6), *Staphylococcus epidermidis* (n=2), *Bacillus cereus* (n=3), *Shigella flexneri* (n=3), *Salmonella* Typhi (n=4), *Pseudomonas aeruginosa* (n=3), *Escherichia coli* (n=4), *Klebsiella pneumoniae* (n=3) et *Vibrio cholerae* (n=2). Les résultats de l'AST ont indiqué que *E. coli* était sensible à 99,4% au céfixime mais seulement 10,7% à la tétracycline, *S. flexneri* était sensible à 91,7% à la gentamicine mais seulement 21,6% à cefuroxime, *S.* Typhi était sensible à 100,0% au céfixime mais seulement 19,1% À la tétracycline, *V. cholerae* était sensible à 88,3% au céfixime mais seulement 32,6% à l'ampicilline, *S. aureus* était sensible à 85,9% au cotrimoxazole mais seulement 16,4% à la tétracycline, et *S. epidermidis* était sensible à 92,4% au cotrimoxazole mais seulement 23,7% à tétracycline. D'autres isolats tels que *K. pneumoniae, P. aeruginosa* et *B. cereus* ont montré différents niveaux de résistance à la tétracycline et à l'ampicilline.
Conclusion: Notre étude indique qu'en dépit de l'acidité du cocktail «Gegemu», il contient des bactéries pathogènes qui dépassaient les seuils microbiens acceptables, le rendant dangereux pour la consommation. De plus, certaines des bactéries isolées ont montré une résistance à la tétracycline et à l'ampicilline, qui sont des antibiotiques courants utilisés dans notre environnement. Il est essentiel d'améliorer la sensibilisation à la santé publique pour répondre et réduire ces risques.

Mots-clés: bactériologiques; Nombre hétérotrophique; «Gegemu»; cocktail; résistance aux antimicrobiens

Introduction:

Consumption of locally made cocktails produced from mixtures of herbs and chemicals has become increasingly prevalent in the society (1,2). These drinks are made without following official regulations pertaining to food safety and are sometimes referred to as artisanal, unrecorded or illegal (2). Consumers may be exposed to dangerous ingredients in these homemade and adulterated alcoholic drinks (3). One of these locally produced drinks that lacks regulated processing and handling is 'gegemu' cocktail. This cocktail is produced from a plant called *Datura stramonium*, a cosmopolitan plant of worldwide distribution (4).

Datura stramonium has been identified to contain psychoactive constituents (5,6,7), and pharmacologically proven as a hallucinogenic plant that causes serious poisoning due to high toxicity level of the plant parts, with the ripe seeds containing highest amounts of alkaloids (8). Consumption of plant-based psychoactive drinks like 'gegemu' cocktail among youths implies that restricting their abuse is ineffective (9). These psychoactive drinks used during adolescence lead to association with antisocial peer groups, which contribute to the risk for cognitive impairments and psychopathology (4,10).

According to Cífková and Krajčovie-

chová (11), consuming large amounts of alcohol from local drinks can increase the risk of stroke, coronary heart disease, and aortic aneurysm. It is a problem that poses great danger to the lives of many individuals, and enormous strain on the economy, healthcare system, and society at large (12).

Apart from the pharmacological effects resulting from 'gegemu' intake, the production, handling, storage and safety of cocktail produced from this plant remain unknown. A concern which may lead to the microbial contamination of unregulated 'gegemu' cocktail is poor hygiene. Contamination by organisms can occur at any point during the production or storage of finished products for public consumption. Around the world, it is known that poor hygiene in developing countries contributes to the proliferation of food pathogens in several local beverages. The origin of bacterial or fungal contaminations during the processing of many local food and beverages in Nigeria is unclear and the prevalence of microorganisms needs further characterization (2).

Besides, microbial fermentation products of local drinks in Nigeria are poorly studied while it has been shown that the microorganisms responsible for the fermentation of traditional drinks may produce other compounds or derivatives, which are harmful to health (13). These traditional beverages often harbor a diverse array of microorganisms, which can have significant implications for public health and safety (14,15).

Some potential contaminants such as *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Bacillus cereus* have been reported as the main causal agents of foodborne outbreaks as well as yeasts and molds, which can cause spoilage and produce mycotoxins, and other foodborne pathogens (16,17,18]. Since "gegemu" cocktail has become a popular beverage enjoyed at social gatherings and celebrations among the youths in the study area and because of the potential food safety risk posed by its consumption, this informed the conduct of this study. The objectives of the study are to determine the physicochemical property and bacterial contamination of 'gegemu' cocktail, isolate and identify the bacteria contaminants using morphological and biochemical test schemes, and determine the antimicrobial susceptibility of the isolated bacteria.

Materials and method:

Study setting and design:

This was a descriptive bacteriological analysis of 'gegemu' cocktail samples prepared from *Datura stramonium.* Because this cocktail is not commercially available, a purposive sampling technique was adopted to collect 10 samples of the 'gegemu' cocktail from students in three selected tertiary institutions within Abeokuta metropolis, Nigeria. The analysis was performed at the biology laboratory of the Federal College of Education Abeokuta, Nigeria in 2023.

Sample collection:

Ten samples of the 'gegemu' cocktail were obtained from the purposively selected students and transported to the laboratory in ice packs for analysis.

Determination of pH of collected samples:

The pH of the 'gegemu' samples was measured in triplicates using a standard pH meter (Model 3510 Complete pH/Mv) after calibrating with buffer solution (pH 7.0). This was done at 12 hourly intervals (19).

Preparation of culture media:

Culture media used for isolation were plate count agar (PCA), nutrient agar (NA), *Salmonella-Shigella* agar (SSA), Eosin Methylene Blue agar (EMBA), and MacConkey agar (MA). All media were prepared according to the manufacturers' instructions and sterilized at 121°C for 15 minutes in an autoclave (20).

Enumeration of bacteria:

One milliliter of 'gegemu' sample was introduced into a test tube containing 9.0 ml of sterile distilled water. Dilutions were made up to 10^{-6} from the stock solution using serial dilution method. One milliliter of the 10^{-5} and 10^{-6} dilution was seeded on the PCA, NA, SSA, EMBA and MA using the pour plate method. The plates were incubated at 37°C for 24 hours. Total colonies on the surface of the plates were counted and expressed as log10 colony forming unit per milliliter (log10 CFU/ml) of the sample (21). This procedure was done in triplicates and the mean (±SD) CFU/ml was determined.

Identification of bacteria:

Identification of isolated bacteria was done using the test methods described in the Bergey's Manual of Systematic Bacteriology (22). The conventional microbiological tests used included Gram staining reaction, motility, catalase, methyl red, oxidase, Voges-Proskauer (VP), citrate utilization, indole, coagulase, and urease tests (23).

Antimicrobial susceptibility testing (AST):

The Kirby-Bauer disk diffusion test was used to determine the antimicrobial susceptibility of the bacterial isolates against 9 selected antibiotics (chosen to ensure unifor-

mity and comparison of susceptibility profiles between isolates and not in a clinical context). The antibiotics tested include cefixime (5µg), tetracycline (10µg), ampicillin (10µg), chloramphenicol (10µg), gentamicin (10µg), cotrimoxazole (25µg), cefotaxime (30µg), ceftriaxone (30µg) and cefuroxime (30µg).

Mueller Hinton (MH) agar plate was inoculated with standardized inoculum (~1x 10^8 CFU/ml) of the test isolates. The antibiotic discs were placed on the MH agar surface and incubated at 37°C for 18-24 hours (24). The diameters of zone of inhibition were measured for each isolate and interpreted as sensitive or resistant according to the Clinical and Laboratory Standard Institutes guideline (25).

Statistical analysis:

Statistical analysis of data used was done using the Statistical Package for the Social Sciences (SPSS) version 16.0 (26). Means were compared using the Duncan's multiple range test (27) and analysis of variance (ANOVA). Significant difference was considered at $p<0.05$.

Results:

The mean pH of the 'gegemu' samples was 3.705±0.005 with a range of 3.25± 0.02 for sample G7 to 4.11±0.02 for sample G5 (Table 1).

Table 1: pH values of 'gegemu' cocktail samples

Sample Code	Mean pH Value
G1	$3.78±0.01^c$
G2	$3.35±0.02^b$
G3	$4.08±0.01^a$
G4	$3.52+0.02^d$
G5	$4.11±0.02^a$
G6	$3.63±0.01^a$
G7	$3.25±0.02^d$
G8	$3.80±0.01^d$
G9	$3.52±0.02^c$
G10	$4.01±0.01^a$
Overall mean pH	**3.705±0.005**

Superscripts a, b, c, d = mean values are significantly different between samples

All the 'gegemu' samples were contaminated by bacteria, with total heterotrophic counts ranging from mean value of 5.8±0.09 x10^5 CFU/ml for sample G4 to 7.9±0.24x10^6 CFU/ml for sample G8, which exceeded the acceptable level of microbial contents of drinks safe for consumption (Table 2).

Table 2: Heterotrophic bacterial counts of 'gegemu' cocktail samples

Sample code	Mean bacterial count (CFU/ml)	
	(x10^5)	**(x10^6)**
G1	$8.1±0.03^c$	$7.7±0.11^b$
G2	$6.7±0.07^a$	$5.6±0.02^d$
G3	$7.4±0.11^b$	$6.2±0.33^c$
G4	$5.8±0.09^b$	$4.1±0.21^a$
G5	$7.4±0.01^c$	$5.6±0.03^b$
G6	$7.7±0.11^a$	$4.9±0.33^c$
G7	$6.8±0.01^b$	$5.2±0.01^b$
G8	$9.2±0.02^a$	$7.9±0.24^c$
G9	$8.7±0.01^c$	$6.6.\ ±0.03^a$
G10	$6.0±0.23^d$	$4.8±0.31^b$

G= 'gegemu'; Superscripts a, b, c, d = mean values are significantly different between samples

A total of 30 bacterial isolates were recovered from the 'gegemu' samples (Table 3). The frequency (in %) of bacteria species isolated shows that *Staphylococcus* species were the most frequent isolates (26.7%), followed by *E. coli* and *S.* Typhi (13.3% each), *K. pneumoniae*, *P. aeruginosa, S. flexneri* and *B. cereus* (10.0% each), and *V. cholerae* (6.7%) was the least frequent.

Table 3: Frequency of bacteria isolated from 'gegemu' cocktail samples

Bacterial isolates	Frequency	Percentage (%)
Staphylococcus spp	8	26.7
Escherichia coli	4	13.3
Salmonella Typhi	4	13.3
Klebsiella pneumoniae	3	10
Pseudomonas aeruginosa	3	10
Shigella flexneri	3	10
Bacillus cereus	3	10
Vibrio cholerae	2	6.7
Total	30	100.0

Table 4: Antimicrobial susceptibility of the bacterial isolates from 'gegemu' cocktail samples

Bacterial isolate	**Number (%) of isolates sensitive to antibiotics**								
	CEF	**TET**	**AMP**	**CHL**	**GENT**	**COT**	**CFX**	**CFR**	**CRX**
Escherichia coli (n=4)	4 (99.4)	4 (10.7)	4 (17.3)	4 (93.2)	4 (82.1)	2 (33.5)	3 (73.4)	2 (77.4)	1 (59.3)
Shigella flexneri (n=3)	2 (87.8)	1 (56.9)	2 (51.1)	3 (78.3)	3 (91.7)	2 (53.8)	1 (62.2)	2 (71.4)	1 (21.6)
Salmonella Typhi (n=4)	4 (100)	4 (19.1)	3 (73.7)	2 (44.8)	4 (76.1)	4 (80.40)	2 (52.3)	4 (100)	2 (64.2)
Vibrio cholerae (n=2)	2 (88.3)	2 (84.2)	2 (32.6)	1 (55.1)	1 (73.5)	1 (66.4)	1 (72.8)	1 (68.7)	2 (79.4)
Staphylococcus aureus (n=6)	5 (83.6)	6 (16.4)	5 (21.2)	5 (31.2)	4 (62.3)	5 (85.9)	5 (84.4)	4 (72.5)	5 (23.1)
Staphylococcus epidermidis (n=2)	2 (81.9)	2 (23.7)	1 (41.2)	2 (72.4)	2 (81.6)	2 (92.4)	2 (81.2)	1 (83.6)	1 (66.7)
Klebsiella pneumoniae (n=3)	2 (86.4)	1 (52.6)	3 (19.2)	2 (23.5)	3 (88.4)	2 (79.2)	2 (68.9)	2 (75.2)	3 (88.7)
Pseudomonas aeruginosa (n=3)	3 (90.4)	3 (13.8)	3 (17.7)	2 (34.6)	3 (74.6)	2 (81.2)	3 (86.4)	2 (81.6)	2 (19.7)
Bacillus cereus (n=3)	2 (86.9)	3 (70.5)	1 (42.3)	2 (84.5)	3 (90.1)	2 (79.5)	3 (72.7)	3 (88.4)	2 (77.6)

CEF: Cefixime (5µg), TET: Tetracycline (10µg), AMP: Ampicillin (10µg), CHL: Chloramphenicol (10µg), GENT: Gentamicin (10µg), COT: Cotrimoxazole (25µg), CFX: Cefotaxime (30µg), CFR: Ceftriaxone (30µg), CRX: Cefuroxime (30µg)

The AST profile of the isolates (Table 4) shows that more than 50% of the *E. coli* isolates were susceptible to cefixime (99.9%), chloramphenicol (93.2%), gentamicin (82.1%), cefotaxime (73.4%), ceftriaxone (77.4%), and cefuroxime (59.3%) but only 10.7% were sensitive to tetracycline, 17.3% to ampicillin and 33.5% to cotrimoxazole. Over 50% of *S. flexneri* isolates were sensitive to cefixime (87.8%), tetracycline (56.9%), ampicillin (51.1%), chloramphenicol (78.3%), gentamicin (91.7%), cotrimoxazole (53.8%), cefotaxime (62.2%), ceftriaxone (71.4%), while only 21.6% were sensitive to cefuroxime. Over 50% of *S.* Typhi isolates were sensitive to cefixime (100%), ampicillin (73.7%), gentamicin (76.1%), cotrimoxazole (80.4%), cefotaxime (52.3%), ceftriaxone (100%), and cefuroxime (64.2%), but only 19.1% were sensitive to tetracycline and 44.8% to chloramphenicol. More than 50% of *V. cholerae* isolates were sensitive to cefixime (88.3%), tetracycline (84.2%), chloramphenicol (55.1%), gentamicin (73.5%), cotrimoxazole (66.4%), cefotaxime (72.8%), ceftriaxone (68.7%), and cefuroxime (79.4%), while only 32.6% were sensitive to ampicillin.

Over 50% of the *K. pneumoniae* isolates were sensitive to cefixime (86.4%), tetracycline (52.6%), gentamicin (88.4%), cotrimoxazole (79.2%), cefotaxime (68.9%), ceftriaxone (75.2%), and cefuroxime (88.7%), but only 19.2% were sensitive to ampicillin and 23.5% to chloramphenicol. *Pseudomonas aeruginosa* isolates were sensitive to cefixime (90.4%), gentamicin (74.6%), cotrimoxazole (81.2%), cefotaxime (86.4%), and ceftriaxone (81.6%), but only 13.8% were sensitive to tetracycline, 17.7% to ampicillin, 34.6% to chloramphenicol, and 19.7% to cefuroxime. *Bacillus cereus* isolates were sensitive to cefixime (86.9%), tetracycline (70.5%), chloramphenicol (84.5%), gentamicin (90.1%), cotrimoxazole (79.5%), cefotaxime (72.7%), ceftriaxone (88.4%), and cefuroxime (77.6%), but only 42.3% were sensitive to ampicillin.

For Gram positive isolates, over 50% of *S. aureus* isolates were sensitive to cefixime (83.6%), gentamicin (62.3%), cotrimoxazole (85.9%), cefotaxime (84.4%), and ceftriaxone (72.5%), but only 16.4% were sensitive to tetracycline, 21.2% to ampicillin, 31.2% to chloramphenicol and 23.1% to cefuroxime (23.1%). Similarly, over 50% of the *S. epidermidis* isolates were sensitive to cefixime (81.9%), chloramphenicol (72.4%), gentamicin (81.6%), cotrimoxazole (92.4%), cefotaxime (81.2%), ceftriaxone (83.6%), and cefuroxime (66.7%), but only 23.7% were sensitive to tetracycline and 41.2% to ampicillin.

Discussion:

This study focused on the physicochemical property, bacteriological quality and antimicrobial susceptibility of bacterial isolates of 'gegemu' cocktail drinks prepared from *D. stramonium* herbal weed and com- monly consumed at social gatherings in our setting. The low pH values (mean value of 3.705± 0.005) recorded in the samples showed that the samples were acidic (pH<7.0). Although this low pH value should reduce the type of bacteria that could be isolated from the samples, some acidophilic bacteria tend to survive (28). Meanwhile, the presence of some bacteria in the samples may alter the pH, thereby allowing other pathogenic bacteria to thrive (29). Aside, the finding of low pH in the samples is a concern for the consumer health because pH value below the value recommended for enamel demineralization (pH 5.5) could cause dissolution and softening of surface tooth structures (erosive potential) of consumers (30).

The bacteriological analyses of the 'gegemu' samples showed the presence of *S. aureus, S. epidermis, E. coli, P. aeruginosa, S.* Typhi*, K. pneumoniae, V. cholerae, S. flexneri* and *B. cereus*. The presence of these bacteria in the samples, which exceeded the acceptable limits, is a pointer to poor hygiene conditions in the preparation, processing or post processing of the 'gegemu' cocktail. These bacteria are potential causative agents of food borne diseases and gastrointestinal infections (31,32).

In this study, most of the bacterial isolates were sensitive to the antibiotics tested, although this observation must be interpreted with caution and generalization of our finding with respect to this cannot be made due to the small number of isolates tested. However, *E. coli, P. aeruginosa* and *S. aureus* were largely resistant to tetracycline and ampicillin, as reported in the literature (33). This is a potential source for transmission of antibiotic resistant bacteria, thereby exposing the consumers to serious health risk. Similarly, almost all the bacterial isolates tested were resistant to ampicillin as documented in the literature (34). Although ampicillin is a first line drug for treating infections caused by these bacteria because of its safety profile, low cost and availability (35), antimicrobial resistance has rendered this drug ineffective. Nevertheless, in combination with other

antimicrobials such as aminoglycosides and β-lactamase inhibitors, efficacy and extended antimicrobial coverage have reduced development of resistance (35,36), and rescued ampicillin from being totally ineffective as an antimicrobial agent.

Conclusion:

Our study showed that 'gegemu' cocktail samples had low pH values and are contaminated by potentially pathogenic bacteria above the acceptable limits, thereby making them unsafe for consumption. Resistance of some of these isolated bacteria to low cost and most available antibiotic, tetracycline and ampicillin, is of public health concern. Hence, the need to regulate the intake of 'gegemu' cocktail drinks in our setting.

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Contributions of authors:

JAO was involved in study conceptualization, execution and manuscript drafting; EM was involved in the study design and manuscript review; ORA was involved in the study execution and data acquisition; OMO was involved in data analysis and interpretation. All authors read and approved the final manuscript submitted for publication.

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No conflict of interest is declared.

References:

1. Motlhanka, K., Zhou, N., and Lebani, K. Microbial and chemical diversity of traditional non-cereal based alcoholic beverages of Sub-Saharan Africa Beverages. 2018; 4 (2): 36. https://doi.org/10.3390/beverages4020036
2. Nwaiwu, O., Aduba, C. C., Igbokwe, V. C., Sam, C. E., and Ukwuru, M. U. Traditional and artisanal beverages in Nigeria: Microbial diversity and safety issues. Beverages. 2020; 6 (3): 53. https://doi.org/10.3390/beverages6030053
3. Okaru, A. O., Rehm, J., Sommerfeld, K., Kuballa T., Walch, S. G., and Lachenmeier, D. W. The threat to quality of alcoholic beverages by unrecorded consumption. In: Grumezescu A. M, Holban A. M (eds). Alcoholic Beverages, Kidlington: Woodhead Publishing, Elsevier; 2019: 1–34.
4. Asiru, M. F., and Abifarin, M. O. Health Promotion through Herb Information: A special discussion of gegemu herb in Yoruba setting of Nigeria. J Health Inform Lib. 2016; 3 (1): 41-45. https://johil.org/index.php/johil/article/view/44
5. Abdurahman, H., Adejumo, O., and Abdulmalik, J. Prevalence of psychoactive substance use among male adolescents in Southwest Nigeria. J Subst Abus. 2019; 24 (5): 475-480. doi:10.1080/14659891.2019.1604840
6. Dumbili, E. W. Drug-related harms among young adults in Nigeria: implications for intervention. J Hum Behav Soc Environ. 2020;30 (8):1013-1029. doi:10.1080/10911359.2020.1790462
7. Dumbili, E. W., Ebuenyi, I. D., and Ugoeze, K. C. New psychoactive substances in Nigeria: a call for more research in Africa. Emerg Trends Drugs Addict Health. 2021; 1(11): 100008. doi:10.1016/j.etdah.2021.100008
8. Oseni, O. A., Olarinoye, C. O., and Amoo, I. A. Studies on chemical compositions and functional properties of thorn apple (*Datura stramonium* L) Solanaceae. Afr J Food Sci. 2011; 5 (2): 40–44.
9. Abasiubong, F., Udobang, J. A., Idung, A. U., Udoh, S. B., and Jombo, H. E. Pattern of psycho active substance use in the northern region of Nigeria. Afr J Drug Alcohol Stud. 2014; 13 (2): 107–115.
10. Hamidullah, S., Thorpe, H. H. A., Frie, J. A., Mccurdy, R. D., and Khokhar, J. Y. Adolescent Substance Use and the Brain: Behavioral, Cognitive and Neuroimaging Correlates. Front Hum Neurosci. 2020;4(14):298. doi:10.3389/fnhum.2020.00298.
11. Cífková, R., and Krajčoviechová, A. Alcohol and cardiovascular disease: Position paper of the Czech Society of Cardiology. Centr Eur J Public Health. 2019; 27: S6-S9. doi:10.21101/cejph.a5998.
12. Onaolapo, O. J., Olofinnade, A. T., Ojo, F. O., Adeleye, O., Falade, J., and Onaolapo, A. Y. Substance use and substance use disorders in Africa: An epidemiological approach to the review of existing literature. World J Psychiatr. 2022; 12 (10): 1268-1286. doi:10.5498/wjp.v12.i10.1268.
13. Nwaiwu, O., and Itumoh, M. Chemical contaminants associated with palm wine from Nigeria are potential food safety hazards. Beverages. 2017; 3: 16. https://doi.org/10.3390/beverages3010016
14. Shayo, N. B., Kamala, A., Gidamis, A. B., and Nnko, S. A. Aspects of manufacture, composition and safety of orubisi: a traditional alcoholic beverage in the north-western region of Tanzania. Int J Food Sci Nutr. 2000; 51 (5): 395-402. doi:10.1080/096374800426993
15. Mwambete, D. K., Justin-Temu, M., Mashurano, M., and Tenganamba, O. Microbial Quality of Traditional Alcoholic Beverages Consumed in Dar es Salaam, Tanzania. East Centr Afr J Pharm Sci. 2006; 9: 8-13. doi:10.4314/ecajps.v9i1.9730
16. Doulgeraki, A. I., Di Ciccio, P., Ianieri, A., and Nychas, G. E. Methicillin-resistant food related *Staphylococcus aureus*: A review of current knowledge and biofilm formation for future studies and applications. Res Microbiol. 2017; 168: 1-15.

doi:10.1016/j.resmic.2016.08.001.

17. Lentz, S. A. M., Rivas, P. M., Cardoso, M. R. I., Morales, D. L., Centenaro, F. C., and Martins, A. F. *Bacillus cereus* as the main causal agent of foodborne outbreaks in Southern Brazil: Data from 11 Years. Cadernos de Saúde Pública. 2018; 34: e00057417. doi:10.1590/0102-311X00057417.
18. Pang, B., Zhao, C., Li, L., et al. Development of a low-cost paper-based ELISA method for rapid *Escherichia coli* O157:H7 detection. Anal Biochem. 2018; 542: 58–62. doi: 10.1016/j.ab.2017.11.010.
19. Taiwo, O. S., Afolabi, R. O., Oranusi, S. U., et al. Microbiological Assessment of Commercial Yogurt Sold in Ota Metropolis, Ogun State, Nigeria. IOP Conf Series. 2018; 210: 1-18. doi:10.1088/1755-1315/210/1/012019
20. Abraham, O. G., Idowu, B. O., Barbara, A. U., Veadams, I. E., and Alexander, E. O. Qualitative Detection and Isolation of Bacteria from Surfaces of Canned Drinks Sold in Ugbor, Benin City. Ann Sci Tech. 2018; 3 (2): 20–25 doi:10.2478/ast-2018-0016
21. Terrones-Fernandez, I., Casino, P., López, A., et al. Improvement of the Pour Plate Method by Separate Sterilization of Agar and Other Medium Components and Reduction of the Agar Concentration. Microbiol Spectr. 2023; 11(1): e0316122. doi:10.1128/spectrum.03161-22
22. Holt, J. G., Krieg, N. R., Sneath, P. H. A., et al. Bergey's Manual of Determinative Bacteriology, Baltimore. 1994.
23. Cheesbrough, M. District Laboratory Practice In Tropical Countries. 2nd ed. Cambridge, United Kingdom: Cambridge University Press. 2006; 38: 62–69.
24. Balouiri, M., Sadiki, M., and Ibnsouda, S. K. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6 (2): 71-79. doi:10.1016/j.jpha.2015.11.005.
25. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 11th edn. CLSI, Wayne, PA; 2012.
26. George, D., and Mallery, P. IBM SPSS Statistics 26 Step by Step: A Simple Guide and Reference 16th ed. New York: Routledge. 2019: 1-402.
27. Duncan, D. B. Multiple Range and Multiple F Tests. Biometrics. 1955; 11 (1): 1-42.
28. Mwambete, K. D., and Peter, A. Microbiological Quality of Juice Beverages Available in Dares Salaam and Resistance Profiles of Microbial Contaminants. East Centr Afr J Pharm Sci. 2011; 14: 81-88.
29. Oladipo, I. C., Adeleke, D. T., and Adebiyi, A. O. The effect of pH and chemical preservatives on the growth of bacterial isolates from some Nigerian packaged fruit juices**.** Pakistan J Biol Sci. 2010; 13: 16-21. doi:10.3923/pjbs.2010.16.21.
30. Quartey-Papafio, N., Odoom, M., Sackeyfio, J., and Tormeti, D. The acidity of some selected soft drinks on the Ghanaian market. Afr J Dent 2019; 7 (6): 001-007.
31. Liu, Z., Lu, Y., Zhang, J., Pardee, K., and Wang, P. G. P1 trisaccharide (Gaα1, 4Ga β1 4GcNAC) synthesis by enzyme gycosylation reactions using recombinant *Escherichia coli*. Appl Environ Microbiol. 2003; 69: 2110-2115. doi:10.1128/AEM.69.4.2110-2115.2003.
32. Anon, S. Consensus development conference statement: Traveler's diarrhoea. Rev Infec Dis. 2004; S223-S227.
33. Ayamah, A., Sylverken, A. A., and Ofori, L. A. Microbial load and antibiotic resistance of *Escherichia coli* and *Staphylococcus aureus* isolated from ready-to-eat (RTE) khebab sold on a university campus and its environs in Ghana. J Food Quality. 2021;1-9. https://doi.org/10.1155/2021/8622903
34. Maqbool, M., Akmal, M. N., and Tahir, A. Antimicrobial resistance and drug profile of ampicillin. Indo Am J Pharm Sci. 2020; 7(3): 687-690. http://doi.org/10.5281/zenodo.3726169
35. Kaushik, D., Mohan, M., Borade, D. M., and Swam, O. C. Ampicillin: Rise Fall and Resurgence. J Clin Diagn Res. 2014**;** 8 (5): ME01–ME03. doi:10.7860/JCDR/2014/8777.4356
36. Katzung, B. G., Masters, S. B., and Trevor, A. J. Basic and Clinical Pharmacology. 12th edn. New Delhi: Tata McGraw Hill; 2012.

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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

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