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Editorial **Open Access**

Reducing the risks of nuclear war—the role of health professionals

In January, 2023, the Science and Security Board of the Bulletin of the Atomic Scientists moved the hands of the Doomsday Clock forward to 90s before midnight, reflecting the growing risk of nuclear war (1). In August, 2022, the UN Secretary-General, António Guterres, warned that the world is now in "a time of nuclear danger not seen since the height of the Cold War" (2). The danger has been underlined by growing tensions between many nuclear armed states (1,3). As editors of health and medical journals worldwide, we call on health professionals to alert the public and our leaders to this major danger to public health and the essential life support systems of the planet, and urge action to prevent it.

Current nuclear arms control and non-proliferation efforts are inadequate to protect the world's population against the threat of nuclear war by design, error, or miscalculation. The Treaty on the Non-Proliferation of Nuclear Weapons (NPT) commits each of the 190 participating nations "to pursue negotiations in good faith on effective measures relating to cessation of the nuclear arms race at an early date and to nuclear disarmament, and on a treaty on general and complete disarmament under strict and effective international control" (4). Progress has been disappointingly slow and the most recent NPT review conference in 2022 ended without an agreed statement (5). There are many examples of near disasters that have exposed the risks of depending on nuclear deterrence for the indefinite future (6). Modernisation of nuclear arsenals could increase risks, for example, hypersonic missiles decrease the time available to distinguish between an attack and a false alarm, increasing the likelihood of rapid escalation.

Any use of nuclear weapons would be catastrophic for humanity. Even a "limited" nuclear war involving only 250 of the 13000 nuclear weapons in the world could kill 120 million people outright and cause global climate disruption leading to a nuclear famine, putting 2 billion people at risk (7,8). A large-scale nuclear war between the USA and Russia could kill 200 million people or more in the near term, and potentially cause a global "nuclear winter" that could kill 5–6 billion people, threatening the survival of humanity (7,8). Once a nuclear weapon is detonated, escalation to all-out nuclear war could occur rapidly. The prevention of any use of nuclear weapons is therefore an urgent public health priority and fundamental steps must also be taken to address the root cause of the problem, by abolishing nuclear weapons.

The health community has had a crucial role in efforts to reduce the risk of nuclear war and must continue to do so in the future (9). In the 1980s, the efforts of health professionals, led by the International Physicians for the Prevention of Nuclear War (IPPNW), helped to end the Cold War arms race by educating policy makers and the public on both sides of the Iron Curtain about the medical consequences of nuclear war. This was recognised when the 1985 Nobel Peace Prize was awarded to the IPPNW (10).

In 2007, the IPPNW launched the International Campaign to Abolish Nuclear Weapons, which grew into a global civil society campaign with hundreds of partner organisations. A pathway to nuclear abolition was created with the adoption of the Treaty on the Prohibition of Nuclear Weapons in 2017, for which the International Campaign to Abolish Nuclear Weapons was awarded the 2017 Nobel Peace Prize. International medical organisations, including the International Committee of the Red Cross, the IPPNW, the World Medical Association, the World Federation of Public Health Associations, and the International Council of Nurses, had key roles in the process leading up to the negotiations, and in the negotiations themselves, presenting the scientific evidence about the catastrophic health and environmental consequences of nuclear weapons and nuclear war. They continued this important collaboration during the First Meeting of the States Parties to the Treaty on the Prohibition of Nuclear Weapons, which currently has 92 signatories, including 68 member states (11).

We now call on health professional associations to inform their members worldwide about the threat to human survival and to join with the IPPNW to support efforts to reduce the near-term risks of nuclear war, including three immediate steps on the part of nuclear-armed states and their allies: first, adopt a no first use policy (12); second, take their nuclear weapons off hair-trigger alert; and, third, urge all states involved in current conflicts to pledge publicly and unequivocally

that they will not use nuclear weapons in these conflicts. We further ask them to work for a definitive end to the nuclear threat by supporting the urgent commencement of negotiations among the nuclear-armed states for a verifiable, timebound agreement to eliminate their nuclear weapons in accordance with commitments in the NPT, opening the way for all nations to join the Treaty on the Prohibition of Nuclear Weapons.

The danger is great and growing. The nuclear armed states must eliminate their nuclear arsenals before they eliminate us. The health community played a decisive part during the Cold War and more recently in the development of the Treaty on the Prohibition of Nuclear Weapons. We must take up this challenge again as an urgent priority, working with renewed energy to reduce the risks of nuclear war and to eliminate nuclear weapons.

References:

1. Science and Security Board, Bulletin of the Atomic Scientists. A time of unprecedented danger: it is 90 seconds to midnight. 2023 Doomsday Clock State- ment. Jan 24, 2023. https://thebulletin.org/doomsday-clock/current-time/ (Accessed June 1, 2023).
2. United Nations. Future Generations Counting on Our Commitment to Step Back from Abyss, Lift Cloud of Nuclear Annihilation for Good, Secretary-General Tells Review Conference, Press Release Aug 1, 2022 SG/SM/21394 https://press.un.org/en/2022/sgsm21394.doc.htm (Accessed 10 July 2023)
3. Tollefson, J. Is nuclear war more likely after Russia's suspension of the New START treaty? Nature. 2023; 615: 386.
4. United Nations. Review Conference of the Parties to the Treaty on the Non-Proliferation of Nuclear Weapons (NPT). May 2–27, 2005. https://www.un.org/en/conf/npt/2005/npttreaty.html (Accessed June 2, 2023).
5. Mukhatzhanova, G. 10th NPT Review Conference: why it was doomed and how it almost succeeded. Arms Control Association. October, 2022. https://www.armscontrol.org/act/2022-10/features/10th-npt-review-conference-why-doomed-almost-succeeded (Accessed June 2 2023).
6. Lewis, P., Williams, H., Pelopidas, B., and Aghlani, S. Too close for comfort, cases of near nuclear use and options for policy. Chatham House Report. April, 2014. https://www.chathamhouse.org/2014/04/too-close-comfort-cases-near-nuclear-use-and-options-policy (Accessed June 1, 2023).
7. Bivens, M. Nuclear famine. IPPNW. August, 2022. https://www.ippnw.org/wp-content/uploads/2022/09/ENGLISH-Nuclear-Famine-Report-Final-bleed-marks.pdf (Accessed June 1, 2023).
8. Xia, L., Robock, A., Scherrer, K., et al. Global food insecurity and famine from reduced crop, marine fishery and livestock production due to climate disruption from nuclear war soot injection. Nat Food 2022; 3: 586–96.
9. Helfand, I., Lewis, P., and Haines, A. Reducing the risks of nuclear war to humanity. Lancet. 2022; 399: 1097–98.
10. Nobel Prize Outreach AB. International Physicians for the Prevention of Nuclear War—facts. 1985. https://www.nobelprize.org/prizes/peace/1985/physicians/facts/ (Accessed June 1, 2023).
11. UN Office for Disarmament Affairs. Treaties Data- base. Treaty on the Prohibition of Nuclear Weapons, status of the Treaty. 2023. https://treaties.unoda.org/t/tpnw (Accessed June 1, 2023.
12. Center for Arms Control and Non-Proliferation. No first use: frequently asked questions. 2023. https://armscontrolcenter.org/issues/no-first-use/no-first-use-frequently-asked-questions/ (Accessed June 2, 2023).

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Editorial **Open Access**

COP28 Climate Change Conference: Time to treat the climate and nature crisis as one indivisible global health emergency

Over 200 health journals call on the United Nations, political leaders, and health professionals to recognise that climate change and biodiversity loss are one indivisible crisis and must be tackled together to preserve health and avoid catastrophe. This overall environmental crisis is now so severe as to be a global health emergency. The world is currently responding to the climate crisis and the nature crisis as if they were separate challenges. This is a dangerous mistake.

The 28th Conference of the Parties (COP) on climate change is about to be held in Dubai while the 16th COP on biodiversity is due to be held in Turkey in 2024. The research communities that provide the evidence for the two COPs are unfortunately largely separate, but they were brought together for a workshop in 2020 when they concluded that; "Only by considering climate and biodiversity as parts of the same complex problem…can solutions be developed that avoid maladaptation and maximize the beneficial outcomes" (1).

As the health world has recognised with the development of the concept of planetary health, the natural world is made up of one overall interdependent system. Damage to one subsystem can create feedbacks that damage another, for example, drought, wildfires, floods and the other effects of rising global temperatures, destroy plant life, and lead to soil erosion and so inhibit carbon storage, which means more global warming (2). Climate change is set to overtake deforestation and other land-use change as the primary driver of nature loss (3).

Nature has a remarkable power to restore. For example, deforested land can revert to forest through natural regeneration, and marine phytoplankton, which act as natural carbon stores, turn over one billion tonnes of photosynthesising biomass every eight days (4). Indigenous land and sea management has a particularly important role to play in regeneration and continuing care (5). Restoring one subsystem can help another, for example, replenishing soil could help remove greenhouse gases from the atmosphere on a vast scale (6). But actions that may benefit one subsystem can harm another, for example, planting forests with one type of tree can remove carbon dioxide from the air but can damage the biodiversity that is fundamental to healthy ecosystems (7).

The impacts on health:

Human health is damaged directly by both the climate crisis, as the journals have described in previous editorials (8,9), and by the nature crisis (10). This indivisible planetary crisis will have major effects on health as a result of the disruption of social and economic systems—shortages of land, shelter, food, and water, exacerbating poverty, which in turn will lead to mass migration and conflict. Rising temperatures, extreme weather events, air pollution, and the spread of infectious diseases are some of the major health threats exacerbated by climate change (11). "Without nature, we have nothing," was UN Secretary-General António Guterres's blunt summary at the biodiversity COP in Montreal last year (12). Even if we could keep global warming below an increase of 1.5ºC over pre-industrial levels, we could still cause catastrophic harm to health by destroying nature.

Access to clean water is fundamental to human health, and yet pollution has damaged water quality, causing a rise in waterborne diseases (13). Contamination of water on land can also have far-reaching effects on distant ecosystems when that water runs off into the ocean (14). Good nutrition is underpinned by diversity in the variety of foods, but there has been a striking loss of genetic diversity in the food system. Globally, about a fifth of people rely on wild species for food and their livelihoods (15). Declines in wildlife are a major challenge for these populations, particularly in low-and-middle-income-countries. Fish provide more than half of dietary protein in many African, South Asian and small island nations, but ocean acidification has reduced the quality and quantity of seafood (16).

Changes in land use have forced tens of thousands of species into closer contact, increasing the exchange of pathogens and the emergence of new diseases and pandemics (17). People losing contact with the natural environment and the decline loss in biodiver-

sity have both been linked to increases in non-communicable, autoimmune and inflammatory diseases, metabolic, allergic, and neuropsychiatric disorders (10,18). For Indigenous people, caring for and connection with nature is especially important for their health (19). Nature has also been an important source of medicines, and thus reduced diversity also constrains the discovery of new medicines. Communities are healthier if they have access to high-quality green spaces that help filter air pollution, reduce air and ground temperatures, and provide opportunities for physical activity (20). Connection with nature reduces stress, loneliness and depression, while promoting social interaction (21). These benefits are threatened by the continuing rise in urbanisation (22).

Finally, the health impacts of climate change and biodiversity loss will be experienced unequally between and within countries, with the most vulnerable communities often bearing the highest burden (10). Linked to this, inequality is also arguably fuelling these environmental crises. Environmental challenges and social/health inequities are challenges that sh- are drivers and there are potential co-benefits of addressing them (10).

A global health emergency:

In December 2022, the biodiversity COP agreed on the effective conservation and management of at least 30% percent of the world's land, coastal areas, and oceans by 2030 (23). Industrialised countries agreed to mobilise $30 billion per year to support developing nations to do so (23). These agreements echo promises made at climate COPs. Yet many commitments made at COPs have not been met. This has allowed ecosystems to be pushed further to the brink, greatly increasing the risk of arriving at 'tipping points', abrupt breakdowns in the functioning of nature (2,24). If these events were to occur, the impacts on health would be globally catastrophic.

This risk, combined with the severe impacts on health already occurring, means that the World Health Organization should declare the indivisible climate and nature crisis as a global health emergency. The three preconditions for WHO to declare a situation to be a Public Health Emergency of International Concern (25) are that the situation; (1) is serious, sudden, unusual or unexpected; (2) carries implications for public health beyond the affected State's national border; and (3) may require immediate international action. Climate change would appear to fulfil all of these conditions. While the accelerating climate change and loss of biodiversity are not sudden or unexpected, they are certainly serious and unusual. Hence, we call for WHO to make this declaration before or at the Seventy-seventh World Health Assembly in May 2024.

Tackling this emergency requires the COP processes to be harmonised. As a first step, the respective conventions must push for better integration of national climate plans with biodiversity equivalents (3). As the 2020 workshop that brought climate and nature scientists together concluded, "Critical leverage points include exploring alternative visions of good quality of life, rethinking consumption and waste, shifting values related to the human-nature relationship, reducing inequalities, and promoting education and learning" (1). All of these would benefit health.

Health professionals must be powerful advocates for both restoring biodiversity and tackling climate change for the good of health. Political leaders must recognise both the severe threats to health from the planetary crisis as well as the benefits that can flow to health from tackling the crisis (26). But first, we must recognise this crisis for what it is: a global health emergency.

References:

1. Otto-Portner, H., Scholes, B., Agard, J., et al. Scientific outcome of the IPBES-IPCC co-sponsored workshop on biodiversity and climate change. 2021. doi:10.5281/zenodo.4659159.
2. Ripple, W. J., Wolf, C., Lenton, T. M., et al. Many risky feedback loops amplify the need for climate action. One Earth. 2023; 6: 86–91. https://www.sciencedirect.com/science/article/abs/pii/S2590332223000040. (Accessed 27 June 2023)
3. European Academies Science Advisory Council. Key Messages from European Science Academies for UNFCCC COP26 and CBD COP15. 2021. https://easac.eu/publications/details/key-messages-from-european-science-academies-for-unfccc-cop26-and-cbd-cop15. (Accessed 15 September 2023)
4. Falkowski, P. Ocean Science: The power of plankton. In: Nature Publishing Group UK. doi:10.1038/483S17a
5. Dawson, N. M., Coolsaet, B., Sterling, E. J., et al. The role of Indigenous peoples and local communities in effective and equitable conservation. Ecology and Society. 2021; 26(3):19.https://doi.org/10.5751/ES-12625-260319
6. Bossio, D. A., Cook-Patton, S.C., Ellis, P.W., et al. The role of soil carbon in natural climate solutions. Nat Sustain. 2020; 3: 391–398. https://doi.org/10.1038/s41893-020-0491-z
7. Levia, D. F., Creed, I. F., Hannah, D. M., et al. Homogenization of the terrestrial water cycle. Nat Geosci. 2020; 13:656–658.doi:10.1038/s41561-020-0641-y
8. Atwoli, L., Erhabor, G. E., Gbakima, A. A., et al. COP27 climate change conference: urgent action needed for Africa and the world. BMJ. 2022; 379: 02459. doi:10.1136/bmj.o2459
9. Atwoli, L., Baqui, A. H., Benfield, T., et al. Call for emergency action to limit global temperature increases, restore biodiversity, and protect health. BMJ. 2021; 374: n1734.https://doi.org/10.1136/bmj.n1734
10. WHO, UNEP, Convention on Biological Diversity. Connecting global priorities: biodiversity and human health: A state of knowledge review. 2015. https://www.cbd.int/health/SOK-biodiversity-en.pdf (Accessed 1 October 2023)
11. Magnano San Lio, R., Favara, G., Maugeri, A., Barchitta, M., and Agodi, A. How antimicrobial resistance is linked to climate change: An overview of two intertwined global challenges. Int J Environ Res Publ Health. 2023; 20: 1681.https://doi.org/10.3390/ijerph20031681
12. Jelskov, U. 'Without nature, we have nothing': UN chief sounds alarm at key UN biodiversity event. UN News. 2022.https://news.un.org/en/story/2022/12/1131422. (Accessed 15 September 2023)

13. WHO. State of the world's drinking water: an urgent call to action to accelerate progress on ensuring safe drinking water for all. 2022. https://apps.who.int/iris/rest/bitstreams/1474551/retrieve (Accessed 25 July 2023)
14. Comeros-Raynal, M. T., Brodie, J., Bainbridge, Z., et al. Catchment to sea connection: Impacts of terrestrial run-off on benthic ecosystems in American Samoa, Marine Pollution Bulletin. 2021; 169: 112530. doi: 10.1016/j.marpolbul.2021.112530
15. IPBES. Assessment report on the sustainable use of wild species. Aug 2022.. https://www.ipbes.net/sustainable-use-assessment
16. Falkenberg, L. J., Bellerby, R. G. J., Connell, S. D., et al. Ocean Acidification and Human Health. Int J Environ Res Publ Health. 2020; 17. doi:10.3390/ijerph17124563
17. Dunne, D. Climate change 'already' raising risk of virus spread between mammals. 2022. https://www.carbonbrief.org/climate-change-already-raising-risk-of-virus-spread-between-mammals/. (Accessed 15 September 2023)
18. Altveş, S., Yildiz, H. K., and Vural, H. C. Interaction of the microbiota with the human body in health and diseases. Biosci Microbiota Food Health 2020; 39: 23–32 doi:10.12938/bmfh.19-023
19. Schultz, R., and Cairney, S. Caring for country and the health of Aboriginal and Torres Strait Islander Australians. Med J Aust. 2017; 207 (1):8–10. doi:10.5694/mja16.00687
20. MacGuire, F., Mulcahy, E., and Rossington, B. The Lancet Countdown on Health and Climate Change - Policy brief for the UK. 2022. https://s41874.pcdn.co/wp-content/uploads/Lancet-Countdown-2022-UK-Policy-Brief.EN.Pdf (Accessed 15 September 2023).
21. Wong, F. Y., Yang, L., Yuen, J. W. M., Chang, K. K. P., and Wong, F. K. Y. Assessing quality of life using WHOQOL-BREF: a cross-sectional study on the association between quality of life and neighbourhood environmental satisfaction, and the mediating effect of health-related behaviours. BMC Publ Health. 2018; 18: 1113. doi: 10.1186/s12889-018-5942-3
22. Simkin RD, Seto KC, McDonald RI, Jetz W. Biodiversity impacts and conservation implications of urban land expansion projected to 2050. Proc Natl Acad Sci U S A. 2022;119:e2117297119. doi:10.1073/pnas.2117297119
23. Secretariat of the Convention on Biological Diversity. COP15: Nations Adopt Four Goals, 23 Targets for 2030 In Landmark UN Biodiversity Agreement. Convention on Biological Diversity. 2022. https://www.cbd.int/article/cop15-cbd-press-release-final-19dec2022. (Accessed 15 September 2023).
24. Armstrong McKay, D. I., Staal, A., Abrams, J. F., et al. Exceeding 1.5°C global warming could trigger multiple climate tipping points. Science. 2022; 377: eabn7950. doi: 10.1126/science.abn7950
25. WHO guidance for the use of Annex 2 of the International Health Regulations (2005). In: World Health Organization https://www.who.int/publications/m/item/who-guidance-for-the-use-of-annex-2-of-the-international-health-regulations-(2005). (Accessed 1 Oct 2023)
26. Australian Government Department of Health, Care A. Consultation on Australia's first National Health and Climate Strategy. In: Australian Government Department of Health and Aged Care. https://www.health.gov.au/news/consultation-on-australias-first-national-health-and-climate-strategy (Accessed 1 October 2023)

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Manual **Open Access**

The CLIMIDSON Manual for Antimicrobial Stewardship Programmes in Nigerian Health Care Facilities

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Executive Summary:

Antimicrobial stewardship (AMS) remains a cornerstone of efforts aimed at improving antimicrobial-related patient safety. It slows the development and spread of antimicrobial resistance (AMR), while helping clinicians to improve clinical outcomes and minimise harm by improving antimicrobial prescribing. AMS programmes (ASPs) are driven through various processes and people. An AMS structure comprises the core elements that should be in place to support the ASP including the AMS team, treatment guidelines, and surveillance of AMR and antimicrobial use (AMU). This manual aims to provide a practical guide to health care facilities in Nigeria and other low-and-middle-income countries, for establishing, implementing and sustaining ASPs, and is structured into 14 sections. Section 1 introduces the subject matter and gives background information on the current situation of AMS in Nigeria. It describes the efforts of the National Antimicrobial Stewardship Working Group (NASWOG), an arm of the Clinical Microbiology and Infectious Diseases Society of Nigeria (CLIMIDSON), in identifying the AMR issues in health care facilities in the country and providing evidence-based recommendations for ASPs. Section 2 describes the goals of AMS and core elements which must be in place for successful and sustainable ASPs. Section 3 presents how a health care facility could start an ASP depending on the size, highlighting the important role of point prevalence survey (PPS) in obtaining baseline data on AMU and prescribing practice in health care facilities, which is useful in developing an action plan. Although management support is key for a successful ASP, the governance of the programme rests with the AMS committee, which composition and size will depend on the level of health care facility. Section 4 describes AMS strategies, which include the core and supplemental strategies. Every hospital should aspire to do at least a core strategy, although it may be convenient to start with other stewardship activities and supplemental strategies. Section 5 describes the antibiotic policy and guidelines, which provide the framework for all AMS activities, and is an effective means of changing behaviour in antimicrobial prescribing. The guidelines should be written by a multidisciplinary team and due consideration must be given to the local antibiotic susceptibility data and the common infectious disease syndromes in the facility or region. Dissemination of the policy and guidelines should be given wide publicity. At the primary health care facilities, where there may be no doctors to prescribe, "standing orders" are used to guide antibiotic prescribing. Section 6 describes the critical importance of stakeholder engagement to a successful ASP. If stakeholders are more informed about AMR issues and ASP, they are better able to positively support the programme. AMS stakeholders will differ from facility to facility but generally include health care facility management, clinicians, pharmacists, nurses, infection prevention and control (IPC) practitioners, clinical microbiologists, other relevant laboratory staff, and patients. The importance of education and training to the successful implementation of AMS is presented in section 7. Health care facilities should provide induction and in-service training to all staff on AMS and IPC. Training objectives should be clear and targets of education and training should include AMS committee and team(s), clinicians, pharmacists, nurses and other health care staff, patients and caregivers, and advocacy and community campaigns. Sections 8 and 9 explain how monitoring and evaluation (M&E) of ASP, and feedback to stakeholders are conducted. Monitoring and evaluation are critical to identifying the impact of intervention measures and opportunities for improvement. This involves the evaluation of the structures, processes and outcomes of ASPs. Sections 10 and 11 delved into the roles of clinical microbiology laboratory support for AMS, and diagnostic stewardship as well as information and communication technology (ICT) in ASPs. The clinical microbiology laboratory should provide quality antibiotic susceptibility testing data, and standard antibiograms periodically to the AMS committee. Sections 12, 13 and 14 enumerated the core elements of outpatient ASP, institutional mentoring in AMS, and system building approach to sustainability of ASP. The recommendations for outpatient AMS in this document apply to either stand-alone clinics and casualties or those located in secondary or tertiary hospitals.

Keywords: Antimicrobial resistance; Antimicrobial stewardship; Implementation; Health care facility; Manual

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Le Manuel CLIMIDSON Pour les Programmes de Gestion des Antimicrobiens dans les Établissements de Santé Nigérians

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Résumé Exécutif:

La gestion des antimicrobiens (AMS) demeure la pierre angulaire des efforts visant à améliorer la sécurité des patients liée aux antimicrobiens. Il ralentit le développement et la propagation de la résistance aux antimicrobiens (RAM), tout en aidant les cliniciens à améliorer les résultats cliniques et à minimiser les dommages en améliorant la prescription des antimicrobiens. Les programmes AMS (ASP) sont pilotés par divers processus et personnes. Une structure AMS comprend les éléments de base qui devraient être en place pour soutenir l'ASP, y compris l'équipe AMS, les directives de traitement et la surveillance de la RAM et de l'utilisation d'antimicrobiens (AMU). Ce manuel vise à fournir un guide pratique aux établissements de soins de santé au Nigeria et dans d'autres pays à revenu faible ou intermédiaire, pour l'établissement, la mise en œuvre et le maintien des ASP, et est structuré en 14 sections. La section 1 présente le sujet et donne des informations générales sur la situation actuelle de l'AMS au Nigéria. Il décrit les efforts du Groupe de travail national sur la gestion des antimicrobiens (NASWOG), une branche de la Société de microbiologie clinique et des maladies infectieuses du Nigéria (CLIMIDSON), pour identifier les problèmes de résistance aux antimicrobiens dans les établissements de santé du pays et fournir des recommandations fondées sur des preuves pour ASP. La section 2 décrit les objectifs de l'AMS et les éléments de base qui doivent être en place pour des ASP réussies et durables. La section 3 présente comment un établissement de santé pourrait démarrer un ASP en fonction de la taille, soulignant le rôle important de l'enquête de prévalence ponctuelle (EPP) dans l'obtention de données de base sur l'UAM et les pratiques de prescription dans les établissements de santé, ce qui est utile pour élaborer un plan d'action. Bien que le soutien de la direction soit la clé du succès d'un ASP, la gouvernance du programme incombe au comité AMS, dont la composition et la taille dépendront du niveau de l'établissement de soins de santé. La section 4 décrit les stratégies AMS, qui comprennent les stratégies de base et supplémentaires. Chaque hôpital devrait aspirer à mettre en place au moins une stratégie de base, bien qu'il puisse être pratique de commencer par d'autres activités de gestion et des stratégies supplémentaires. La section 5 décrit la politique et les directives en matière d'antibiotiques, qui fournissent le cadre de toutes les activités de l'AMS et constituent un moyen efficace de modifier les comportements en matière de prescription d'antimicrobiens. Les lignes directrices doivent être rédigées par une équipe multidisciplinaire et une attention particulière doit être accordée aux données locales de sensibilité aux antibiotiques et aux syndromes de maladies infectieuses courants dans l'établissement ou la région. La diffusion de la politique et des lignes directrices devrait faire l'objet d'une large publicité. Dans les établissements de soins de santé primaires, où il se peut qu'il n'y ait pas de médecins à prescrire, des « ordonnances permanentes » sont utilisées pour guider la prescription d'antibiotiques. La section 6 décrit l'importance cruciale de l'engagement des parties prenantes pour la réussite d'un ASP. Si les parties prenantes sont mieux informées sur les problèmes de résistance aux antimicrobiens et sur l'ASP, elles sont mieux à même de soutenir positivement le programme. Les parties prenantes de l'AMS différeront d'un établissement à l'autre, mais comprennent généralement la direction de l'établissement de soins de santé, les cliniciens, les pharmaciens, les infirmières, les praticiens de la prévention et du contrôle des infections (IPC), les microbiologistes cliniques, d'autres membres du personnel de laboratoire concernés et les patients. L'importance de l'éducation et de la formation pour une mise en œuvre réussie de l'AMS est présentée dans la section 7. Les établissements de soins de santé doivent fournir à tout le personnel une formation initiale et continue sur l'AMS et l'IPC. Les objectifs de la formation doivent être clairs et les cibles de l'éducation et de la formation doivent inclure le comité et les équipes AMS, les cliniciens, les pharmaciens, les infirmières et autres personnels de santé, les patients et les soignants, ainsi que les campagnes de plaidoyer et communautaires. Les sections 8 et 9 expliquent comment le suivi et l'évaluation (S&E) de l'ASP et les retours d'information aux parties prenantes sont effectués. Le suivi et l'évaluation sont essentiels pour identifier l'impact des mesures d'intervention et les possibilités d'amélioration. Cela implique l'évaluation des structures, des processus et des résultats des ASP. Les sections 10 et 11 se sont penchées sur les rôles du soutien du laboratoire de microbiologie clinique pour l'AMS, et de la gérance du diagnostic ainsi que des technologies de l'information et de la communication (TIC) dans les ASP. Le laboratoire de microbiologie clinique doit fournir périodiquement au comité AMS des données de test de sensibilité aux antibiotiques de qualité et des antibiogrammes standard. Les sections 12, 13 et 14 énumèrent les éléments de base de l'ASP ambulatoire, le mentorat institutionnel dans l'AMS et l'approche de construction de système pour la durabilité de l'ASP. Les recommandations pour l'AMS ambulatoire dans ce document s'appliquent soit aux cliniques autonomes et aux blessés, soit à celles situées dans des hôpitaux secondaires ou tertiaires.

Mots clés: Résistance aux antimicrobiens; Gestion des antimicrobiens; Mise en œuvre; Programme; Manuel

Section 1: Introduction

1.1. Purpose, Objective and Scope

Purpose:

This document provides evidence-based support and guidance to promote the successful establishment, implementation and sustenance of AMS programmes (ASPs) across Nigerian health care facilities as a strategy to optimise antimicrobial use (AMU) and combat antimicrobial resistance (AMR).

Objective:

To provide a practical guide to health care facilities for establishing, implementing and sustaining ASPs.

Scope:

This manual covers practical steps and guidance on establishing ASPs in primary, secondary and tertiary health care facilities, particularly concerning the promotion of appropriate prescribing and use of antibiotics in health care facilities.

1.2. Background information on AMR and AMS

Antimicrobial agents remain valuable in humanity's fight against infections, and this value is reducing and under grave threat, mainly due to the growing menace of AMR. The large number of such antimicrobial agents discovered in the early twentieth century heralded the golden age of modern medicine (1). These agents have the ability to inhibit or kill microorganisms, hence the concept of AMR connotes the ability of such microorganisms to defeat the actions of antimicrobial agents (2). This translates into a reduction in the efficacy of the agents and a corresponding decrease in positive patient outcomes (2). Aside from increasing mortality, AMR also adds to economic losses, estimated at 100 trillion US dollars in gross domestic products (GDP) globally by 2050 (3) as well as psychosocial stress in treating or controlling infections in patients, caregivers and the health system (4).

Antimicrobial resistance is a naturally occurring phenomenon that is a survival mechanism for wild type bacterial strains. The rate and variety of mechanisms, and the number of resistant microorganisms has increased exponentially in the last couple of decades beyond that which can be wholly ascribed to nature (4). This has been occurring in tandem with a decrease in the discovery, development and adoption of novel antimicrobials (2). The grave implications this has for health care, a world where no effective agents are left to manage even the simplest of infections, was recognised by the WHO in its 2015 action plan to tackle AMR (5,6).

Since the early recognition of penicillin resistance among *Staphylococcus aureus* and the emergence of more resistant types such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA), other resistance types have been discovered and reported to cause high mortality in patients infected with multidrug resistant (MDR) organisms (1,7). Other clinically significant resistant organisms and mechanisms include extended spectrum beta-lactamase (ESBL)-producing *Enterobacterales*, carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem resistant *Pseudomonas aeruginosa* (CRPA), carbapenem-resistant *Enterobacterales* (CRE), vancomycin-resistant *Enterococcus faecium* (VRE), fluoroquinolone-resistant *Campylobacter* (FRC) spp, fluoroquinolone-resistant *Salmonella* (FRS) spp, and cephalosporin-resistant *Neisseria gonorrhoeae* (CRNG) (7,8,9). Additionally, about 4.1% of newly diagnosed infections are caused by multidrug-resistant organisms (MDROs), which are reported to be spreading globally due to increased travels (10).

A recent systematic analysis suggests that in sub-Saharan Africa, AMR contributes up to 27.3 deaths per 100,000 population, and 250,000 deaths were directly attributed to AMR in 2019 alone (7). Nigeria has not been spared the increase in AMR among clinical isolates. Available data show that MRSA rates in clinical infections have reached 50% while resistance to third-generation cephalosporins exceeds 70% among clinical *Escherichia coli*, *Klebsiella* spp and *Pseudomonas aeruginosa* isolates (11,12). While the increased emergence of AMR has multifaceted roots, human activity is by far the most significant factor (13). This activity is in the form of antibiotic misuse, the emergence and effects of which were predicted by Sir Alexander Fleming, who discovered penicillin, as far back as 1945 (14). Animal husbandry and pisciculture comprise the bulk of antibiotic use and may be the major source of resistant bacteria entering the human population. However, a more direct association has been reported between inappropriate prescribing and poor dispensing practices and antimicrobial resistance (4,10,15). These are particularly applicable in low-and-middle-income-countries (LMICs) with limited resources and poor drug regulatory mechanisms (10,13,16). Data have shown that the levels of antibiotic prescribing in Nigeria are high, and vary from one region to another, ranging from 62.4% to 83.5% (17-21). These high prescribing rates are associated with low rates of appropriate prescribing practice (17).

Antimicrobial stewardship (AMS) is defined as the coordinated interventions designed to improve and measure the appropriate use of antimicrobial agents by promoting the selection of the optimal antimicrobial drug regimen including dosing, duration of therapy, and route of administration, thus preventing harm to the patients and preserving drugs for future patients. This was first applied as a term by McGown and Gerding (22), and has been demonstrated as an effective tool to reduce the high levels of inappropriate antimicrobial use, and by extension reverse AMR emergence (6,16, 23). However, the level of awareness of AMS in Nigeria is low, ranging between 28.2% and 40.6% among physicians (24,25), and implementation rate is even lower, with only 10% of health care institutions surveyed having some form of AMS activity (12). The reasons for these include low utilization of microbiology laboratory services by physicians, poor knowledge and awareness among

key stakeholders, and institutional resistance to AMS due to poor knowledge and lack of understanding of AMS strategies (26,27).

The current poor state of AMS practice in our health care facilities emphasises the need for strategies to encourage the adoption of AMS in all aspects of human health care services (16). A sustained engagement of relevant stakeholders is needed to enable AMS programmes to have any chance of success (2,6). One way of engaging effectively is by acquiring data on antibiotic prescribing and utilization within individual health care facilities using acceptable scientific methods such as point prevalence surveys (6,27). In light of this dire need for quality and patient safety in our health care environment, the Clinical Microbiology and Infectious Diseases Society of Nigeria (CLIMIDSON), through its National Antimicrobial Stewardship Working Group (NASWOG), undertook to drive the process of raising awareness and knowledge base aimed at encouraging our health care facilities to embrace AMS practices and make them routine in their daily practice. This manual is one of the tools designed to achieve this purpose. We emphasise that this manual is not exhaustive, but we believe it will help resource-constrained health care facilities to initiate, establish and sustain effective and efficient ASPs.

1.3. National Antimicrobial Stewardship Working Group (NASWOG)

1.3.1. Role of NASWOG:

The National Antimicrobial Stewardship Working Group (NASWOG) is an arm of the Clinical Microbiology and Infectious Diseases Society of Nigeria (an Incorporated Trustee with the Corporate Affairs Commission of Nigeria), charged with the responsibility of promoting AMS in Nigeria. It is a research-led group of health care professionals with experience and zeal for quality health care, who aim to promote antimicrobial stewardship and related practices via mutual understanding, collaborative activities and interdisciplinary health care education. The objectives of NASWOG are to foster an understanding of AMS, enhance knowledge, provide templates for guidelines, educate policy makers and influence national policy.

The group was formally inaugurated in Owerri, Imo State, Nigeria, in August 2018. The inauguration paved way for the first workshop in Owerri, and a communique was published thereafter (28). The membership of NASWOG was drawn mainly from hospitals conducting the Global Point Prevalence Survey (Global-PPS) of AMU and AMR in their hospitals. In attendance were health care workers from various specialities and different parts of the country.

1.3.2. Vision of NASWOG:

To have every health care facility and practitioner in Nigeria practice AMS.

1.3.3. Mission of NASWOG:

To halt and reverse the rising incidence of AMR in Nigeria's health care facilities especially at the tertiary and secondary levels.

1.3.4. Goals of NASWOG:

- Development of national relevant working documents such as antibiotic guidelines, antibiotic policies, and AMS manual.
- Sensitisation and education of stakeholders
- Working with other stakeholders on monitoring antimicrobial consumption in Nigeria
- Establishing and sustaining AMS in Nigerian health care facilities
- Sensitisation of the Nigerian public to the danger of misuse and abuse of antimicrobials

Section 2: Goals and core elements of AMS

2.1. Goals of AMS:

The overarching goals of AMS are;

- Optimise antimicrobial prescribing by ensuring that only those who need antibiotics receive the right drug promptly in the appropriate dose and duration.
- Prevent antimicrobial overuse, misuse and abuse, and development of antimicrobial resistance by ensuring that antibiotic prescribing is evidence-based and indicated. This ensures that those who do not need antibiotics do not have them, and that those who need them do not use them indiscriminately.
- Reduce antibiotic-related adverse effects by ensuring that patients are not given drugs to which they are allergic. Also, that the antibiotic prescribing is evidence-based and as much as possible limited to non-toxic drugs and for an appropriate duration
- Decrease mortality, morbidity and length of hospital stay: When patients are placed on antibiotics without laboratory evidence, it increases the chance of inappropriate therapy, and this in turn increases the likelihood of prolonged illness, extended stay in the hospital, and death.
- Reduce healthcare-associated costs: Very often patients are prescribed ex-

pensive antibiotics without any laboratory evidence supporting that, whereas equally effective and much less expensive alternatives would have been identified for use had there been a laboratory culture. Again, when patients spend more time in the hospital receiving excessive drugs, some of which are unnecessary, they incur more costs.

2.2. Core Elements of AMS:

The core elements of AMS according to the Centers for Disease Control and Prevention (29) are:

- Leadership commitment: Requires the hospital Management recognising the importance of AMS and making a documented commitment, providing resources and taking other actions to support and promote AMS in their facilities.
- Accountability: Requires that ASP needs a leader that is passionate and knowledgeable in AMS issues, who should be responsible for the programme management and outcomes.
- Pharmacy expertise: Requires a passionate clinical pharmacist, preferably one with expertise in infectious diseases.
- Action: Refers to specific interventions taken to address the deficits in practice that promote AMR.
- Tracking: Every action/intervention leads to an outcome, which needs to be measured to determine its impact. This is the monitoring and evaluation component of ASP.
- Reporting: Data from AMS actions need to be shared with all stakeholders. This encourages further participation, cooperation and support
- Education: Necessary education needs to be provided to stakeholders to enable them play their roles effectively in AMS.

Section 3: How to Start an AMS programme (ASP)

In order to start an effective ASP, it is important to obtain baseline information on antimicrobial prescribing and use in health care facilities. This can be done with a simple and objective method, the Point Prevalence Survey (PPS), which can readily be conducted using the Global-PPS method.

In low-and-middle-income-countries (LMICs) such as Nigeria, AMS strategies may be cumbersome in resource-limited settings especially those without good laboratories. Global-PPS provides an easy tool to obtain background data on antimicrobial prescribing, which are necessary for health care facilities to begin their ASP. It also allows the identification of antimicrobial prescribing problems, highlights peculiar issues, and enables monitoring and evaluation of the ASP. AMS programme can be initiated by the appointment of an AMS committee or team (by the hospital management), which is led by a health care practitioner with high level training, interest and passion for AMS/AMR.

3.1. Starting AMS Programme using Global-PPS

The Global-PPS is a simple, standardised, freely available web-based surveillance method (available free at https://www.global-pps.com/) which enables assessment of the quality of antibiotic use and identification of targets for quality improvement. It provides an objective snapshot of the antimicrobial situation at the health care facility at a precise time. The method collects documented data from the medical records on:

- Drug name and dosage of antimicrobials prescribed for the patient
- Diagnosis
- Indications for prescribing antimicrobials
- Route of administration of antimicrobials
- Stop/review date of antimicrobials
- Targeted or empirical therapy
- Availability and compliance with antibiotic guidelines
- Duration of surgical antibiotic prophylaxis
- Healthcare-associated infections
- Presence of invasive devices

3.1.1. Procedures for conducting Global-PPS:

- Register the health care facility (HCF) on the Global-PPS website. This should be done by the AMS committee or/ team lead.
- Assess the HCF to know the following:
    - Number of wards
    - Number of beds
    - Number of patients on admission in each ward
    - Hospital work schedule (clinic, theatre and ward round days)
- Plan on how to carry out the Global-PPS based on the above information
- Identify and engage key stakeholders (department/unit/ward heads) to facilitate the Global-PPS
- Download the information-guiding tools from the Global-PPS website such as

the Global-PPS protocol, data collection forms, and gather other materials such as pencils and erasers needed for the work.
- Assign duties/roles (data collection, validation and entry) to members of the Global-PPS team and train them on the Global-PPS method.
- Collect the data based on the Global-PPS method as detailed on the forms.
- Ensure the data forms are securely kept and available for validation.
- Validate the data by cross-checking for errors and omissions.
- Upload the data onto the web-based Global-PPS analysis tool through the facility portal
- Download analysed data
- Interpret the data and identify key areas for intervention.

3.1.2. Dissemination of Global-PPS data:

The Global-PPS data should be disseminated locally, and probably, nationally and internationally.
- Communicate findings from the Global-PPS to the stakeholders with emphasis on gaps/challenges determined from the Global-PPS data
- When disseminating the data, consider the proposed audience and present the data in a way that enables them to reflect on their practice and change their behaviour.
- Avenues for dissemination of information include;
    - Multi-disciplinary meetings – Grand rounds
    - Department/ward/unit meetings
    - Clinical meetings
    - Presentations at professional meetings and conferences
    - Scientific journals, newsletters and similar publications

3.2. Writing up an AMS action plan:

The AMS action plan (see template at www.climidson.org.ng) should be created based on the findings of the health care facility strength, weakness, opportunity and threat (SWOT) analysis using the CLIMIDSON Assessment checklist (www.climidison.org.ng), which covers the AMS core elements as thematic areas;

- HCF/Leadership/Management
- Commitment
- Accountability
- Pharmacy/Drug Expertise
- Action
- Tracking
- Reporting
- Education

3.3. Advocacy for health facility leadership/management support for AMS

The results of SWOT analysis and Global-PPS data should be used to engage management to; identify AMS as a top priority, provide financial support and dedicated time for health care workers involved in AMS activity, and include AMS responsibilities in job descriptions and appraisal of relevant staff.

3.4. Setting up an Antimicrobial Stewardship Committee and Team(s) with Clear Terms of Reference

3.4.1. Setting up an AMS Committee:

Antimicrobial stewardship programmes (ASPs) require appropriate oversight and governance to ensure proper implementation, monitoring and sustainability. Planning and implementing an effective AMS programme requires the involvement of key players such as facility leadership (management and executives), medical doctors, nurses, pharmacists, microbiologists, and other relevant staff.

The AMS committee should be constituted and inaugurated by the hospital management to coordinate the implementation and review of the AMS programme at the hospital or facility. The AMS committee will bring together key players and involve them in the programmes' decision making from planning to delivery of its initiatives.

3.4.2. Membership of AMS Committee

The membership of the Committee may include;
- A representative from top management
- Clinical microbiologist
- Infectious diseases physician
- Pharmacist
- Nursing representative(s)
- Medical staff representatives from different wards/departments/units (Medicine, Surgery, Obstetrics and Gynaecology, Paediatrics, Oncology, Emergency, Intensive Care, Community Medicine, General Outpatients, Dentistry)
- Drug and Therapeutics Committee representative
- Infection Prevention Control Committee representative
- Clinical microbiology laboratory representative
- Information and Technology (IT) representative (for example, a system analyst)
- Patient Safety and Clinical Quality

Manager
- Consumer representative (optional, if available)
- Medication Safety Committee representative (optional, if available)
- Other personnel may be co-opted as required to assist the work of the Committee.

Membership at secondary and primary health care facilities will depend on available human resources.

3.4.3. AMS Committee Chairman:

The Chairman of the AMS Committee should be a physician with interest, knowledge, passion and expertise in the field of AMS and AMR, usually a Clinical Microbiologist or Infectious Disease Physician (29,30). In their absence, a Clinical Pharmacist or Nurse with the above attributes may play this role.

The key functions of the Chairman are to; directly oversee the activities of the AMS Committee, convene meetings quarterly or more frequently if needed, report on the committee's activities to the management, and represent the committee at meetings amongst others.

3.4.4. Terms of reference of the AMS Committee:

The Committee should have clearly defined Terms of Reference including but not limited to:
- Roles and responsibilities
- Reporting lines
- Review of membership and terms of reference as needed
- Evaluation and identification of Key Performance Indicators (KPI)

3.4.4.1. Roles and Responsibilities
- Reviewing hospital AMS core elements checklist and undertaking SWOT analyses
- Implementing the restriction of selected antimicrobial agents in liaison with the hospital Drug and Therapeutics Committee
- Developing, endorsing and planning implementation of;
    - Systems to review antimicrobial prescribing and feedback results to prescribers
    - Systems to monitor antimicrobial usage and resistance
    - Clinical guidelines for antimicrobial prescribing
    - An education program for good antimicrobial prescribing practice and AMR, in liaison with clinical educators in the hospital
    - Resources to support point-of-care interventions
- Liaising with clinical microbiology services in hospital to ensure selective reporting of susceptibility testing results is in place and aligns with the antimicrobial formulary and guidelines
- Monitoring the effectiveness of strategies used in the ASP at the hospital, including the review of relevant reports and key performance indicators
- Performing AMR surveillance
- Planning action to improve the effectiveness of the ASP at the hospital
- Provide an action plan for the ASP and review it periodically

3.4.4.2. Reporting lines:

The AMS Committee should meet at least quarterly to generate a report of its activities to be presented to the management of the health care facility on a regular basis. The committee can meet as often as it may deem fit in case of emergency. The report should be prepared to outline:

- AMS strategies implemented and progress made, following gap analyses and risk assessments.
- Reports reviewed by the committee relevant to the ASP for example, AMS Team reports, antibiograms, Global-PPS reports

The AMS Team(s) should report to the AMS Committee

3.4.4.3. Evaluation and Key Performance Indicators (KPI):
- The AMS Committee should develop KPIs for the hospital based on the content of the AMS action plan.
    - Process measures from Global-PPS and audit data, for example, the prevalence of AMU.
    - Outcome measures, for example, AMR rate, length of hospital stay and mortality rate.
- Monitoring hospital AMS action plan and its performance.
- Publication and distribution of hospital AMS Committee reports.
- Meeting frequency and attendance in accordance with the terms of reference

3.4.5. AMS Team:

The AMS Team should consist of staff

with daily duties to support the ASP, implement the hospital AMS action plan and facilitate optimised use of antimicrobials in the departments and wards. Depending on the size of the hospital, there can be one AMS Team for the whole hospital (Central AMS Team) and/or an AMS Team for each department/unit.

3.4.5.1. Membership of AMS Team(s):

At the minimum, the Central AMS Team should be led by a physician with an interest in infections or antimicrobial use, and should include a pharmacist and a nurse. At the departmental level, a clinician in that department, preferably the representative of the department in the AMS Committee, should lead the AMS Team.

The departmental AMS Team should comprise mainly doctors and nurses in the department with support from the Central AMS Team and AMS Committee. The AMS Team should be accountable to the hospital AMS Committee.

3.4.5.2. Roles and responsibilities of AMS Team:

- Development, use and review of local antimicrobial guidelines and audit tools
- Promotion of AMS core and supplemental strategies
- Conduct AMS rounds in wards
- Perform monitoring and evaluation of AMS interventions
- In collaboration with the hospital pharmacy, monitor, analyse and interpret the quantity and types of antibiotic use at the unit and/or facility-wide level (antimicrobial consumption data)
- In collaboration with microbiology laboratory, monitor antibiotic susceptibility and resistance rates for a range of key indicator bacteria at the facility-wide level
- Facilitate the required education and training on AMS in the hospital and/or departments.
- Should meet on a regular basis at a frequency that enables them to carry out their responsibilities.

The AMS Team should work in close synergy with IPC and other relevant committees in the facility.

Section 4: Antimicrobial Stewardship Interventions

Antimicrobial stewardship (AMS) interventions comprise actions (or strategies) designed to achieve the objective/goals of the programme. These are divided into two main groups, namely the core and supplemental strategies.

The strategies are normally practised as a bundle of interventions, however, if properly practised, each of these interventions will result in optimising antibiotic use and reducing health care expenditures without compromising clinical outcomes.

4.1. Core strategies:

These are the two-core evidence-based strategies for hospital ASPs. They both require that antibiotic guidelines are obeyed and personnel are available to ensure this. These core strategies enable health care facilities to take direct control over antimicrobial prescribing at the facilities and have the advantage of providing education to prescribers when enquiries are made. The major challenge is that prescribers may perceive a loss of autonomy, especially with pre-authorisation, although this may be overcome with education.

4.1.1. Prospective audit with intervention and feedback:

This is an audit of antibiotic prescribing based on antibiotic guidelines and the hospital antibiotic policy. Recommendations (feedback) are made to the clinicians in real time when the antimicrobial prescribing is considered inappropriate. Recommendations may include changing antibiotics, adjusting doses, duration, and de-escalation based on antibiotic policy and guidelines.

The AMS Team will give feedback to the clinicians, and then check for the level of compliance to the recommendations, and also find out why some may not have complied with the recommendations. The feedback could be during ward rounds, face-to-face meeting, phone conversations, or any other appropriate and effective means. Audit data are obtained from case notes, drug prescription charts or relevant section in the electronic medical record (EMR) where this is used.

4.1.2. Pre-authorisation with formulary restriction:

Under formulary restriction and pre-authorisation, specified antimicrobials will only be dispensed based on approval of prescription by recognised personnel. The choice of restriction to antibiotics may be guided by the WHO AWaRe classification of antimicrobials (31), and this should be clearly specified in the locally formulated antibiotic guidelines.

4.2. Supplemental strategies

4.2.1. Education of health care professionals:

The health care professionals to be

educated should include the AMS Committee, AMS Team(s), clinicians, pharmacists, nurses, IPC practitioners and all other health care workers.

Education has been shown to be impactful in improving antimicrobial prescribing and use (32,33). The goal of education is to achieve behavioural change with respect to antimicrobial prescribing, dispensing, procurement and distribution. Appropriate and standardised content and curricula for health care workers education and training on AMR/AMS have been developed and published (34). The content should create awareness of:

- The impact and drivers of AMR
- Principles and practice of AMS
- Rational or appropriate AMU
- Diagnostic stewardship/surveillance of AMR
- Infection prevention and control

4.2.2. Guidelines and clinical pathways:

Clinical pathways are a common component of improvement initiatives. They aim to organise and standardise care processes, thus maximising patient outcomes and improving organisation efficiency. They may be developed as paper-based forms or charts or algorithm, or electronic documents, and should be based on local protocols (Fig 1, Table 1).

4.2.3. Intravenous (IV) to oral switch therapy:

In many instances there is no advantage of IV antimicrobials over oral formulations except when a high serum concentration is urgently required as in life-threatening conditions such as sepsis and meningitis. Therefore, whenever a patient is able to tolerate orally, an IV or parenteral antimicrobial should be changed to oral, provided there is an oral formulation. This reduces the length of hospital stay so that patients can complete oral antibiotics at home where necessary, and reduces cost and complications associated with the administration of IV antibiotics. There should be a section in the policy and guidelines for IV to oral switch.

4.2.4. Dose optimisation:

Optimised antimicrobial dose should be based on patients' age, weight, organ dysfunction and tissue penetration and other factors such as obesity or critical illness, site of infection (for example central nervous system and blood) and pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the drug, for example, concentration or time dependent activity. For instance, in critically-ill patients, extended-infusion administration of beta-lactams is required for dose optimisation.

Prescribers need to do a review of patient's antimicrobial prescription(s), to determine the need or otherwise to continue the antimicrobial or modify as necessitated by the microbiology laboratory report. The review is usually done after 48-72 hours of administration of empirical therapy, when the laboratory report would have been received.

4.2.5. Automatic stop orders:

This is used to ensure that the administration of specific and prescribed antimicrobials does not exceed a predetermined duration, for example, a maximum of 48 hours for vancomycin where MRSA infection is suspected until confirmed by the laboratory. The

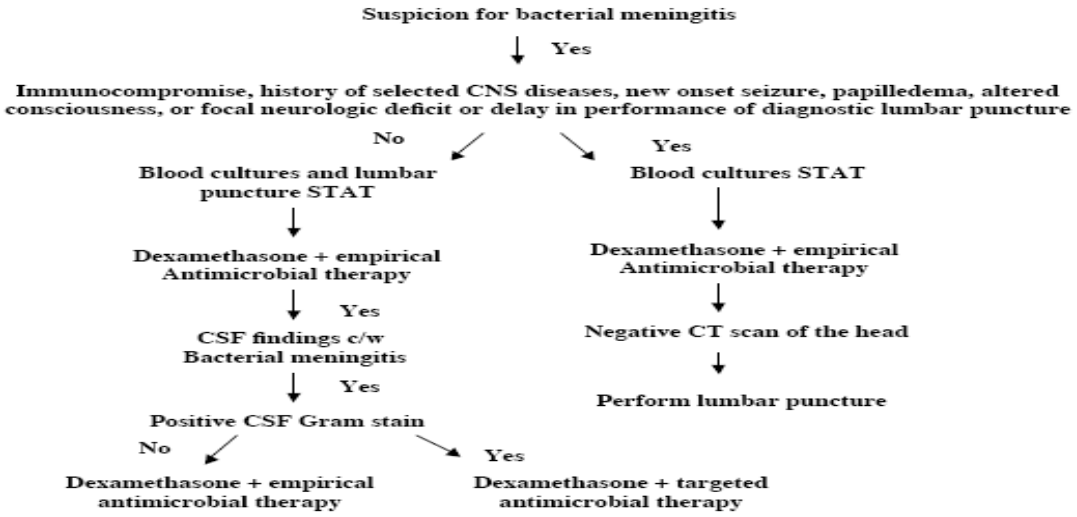

Fig 1: Management algorithm/clinical pathway for bacterial meningitis

Table 1: Template for empirical treatment guideline for bacterial meningitis

Infection syndrome (e. g. Bacterial meningitis)	First line antibiotics (dose/duration)	Alternative antibiotics* (dose/duration)
Age		
0-4 weeks		
1-3 months		
3 months – 50 years		
> 50 years		
Impaired cellular immunity		
Head trauma, neurosurgery		

*May be required for patients with allergies, recent antibiotic therapy or risk factors for specific microorganism

pharmacist stops further dispensing until laboratory evidence is provided confirming the infection requiring the antibiotics. It has the advantage of ensuring that patient safety is not compromised.

4.2.6. De-escalation or escalation:

This refers to the process of changing a prescription from a broad-spectrum antibiotic to a narrower spectrum antibiotic that targets specific microorganisms. Sometimes, escalation to an active antibiotic may be indicated if the identified organism is resistant to the antibiotic in use. Both escalation and de-escalation depend on result of antimicrobial susceptibility test (AST), the type and site of infection.

4.2.7. Microbiology strategy:

4.2.7.1. Comments in Clinical Microbiology Reports:

The clinical microbiologist should provide guidance to prescribers on the interpretation and use of microbiology reports as a routine practice, for example, to know which pathogens might represent colonisation or contamination.

4.2.7.2. Antibiotic testing policy:

- Microbiology laboratories should have a testing policy based on hospital antibiotic guidelines or the WHO AWaRe classification (31). This will ensure doctors do not prescribe antibiotics outside of the hospital formulary.
- Every Clinical Microbiology laboratory should choose antibiotics to test against particular bacteria from clinical samples. This should be in accordance with the facility's antibiotic guidelines and patients' clinical information. This will further guide prescribers in making rational choice of antibiotics.

Section 5: Writing Antibiotic Policy and Guidelines

Antimicrobial stewardship interventions rely on policies and guidelines which provide the basis/framework for all activities. An AMS programme requires the development and use of certain documents which include antibiotic policy and guidelines. These documents should outline how the health care facility would ensure appropriate use of antibiotics and monitor compliance.

Using antibiotic policy and guidelines has been shown to improve patient outcomes, enhance cost savings by reducing the cost of patient care, improve quality of care, reflect local patterns of resistance, and is an effective means of changing behaviour in antimicrobial prescribing.

Antibiotic policy contains the principles that guide rational and prudent antimicrobial prescribing while antibiotic guidelines are recommendations for prescribing antimicrobials for specific indications.

5.1. Objectives of Antibiotic Policy and Guidelines

- To promote appropriate use of antimicrobials.
- To provide a simple approach for treating common infections.
- To curb the emergence of AMR in the health care facility.
- To recommend antimicrobials with consideration of local antibiotic suscep ptibility patterns.

5.2. Writing the Policy and Antibiotic Guidelines

The flow chart of the process of writing antibiotic policy and guidelines is shown in Fig 2 (35).

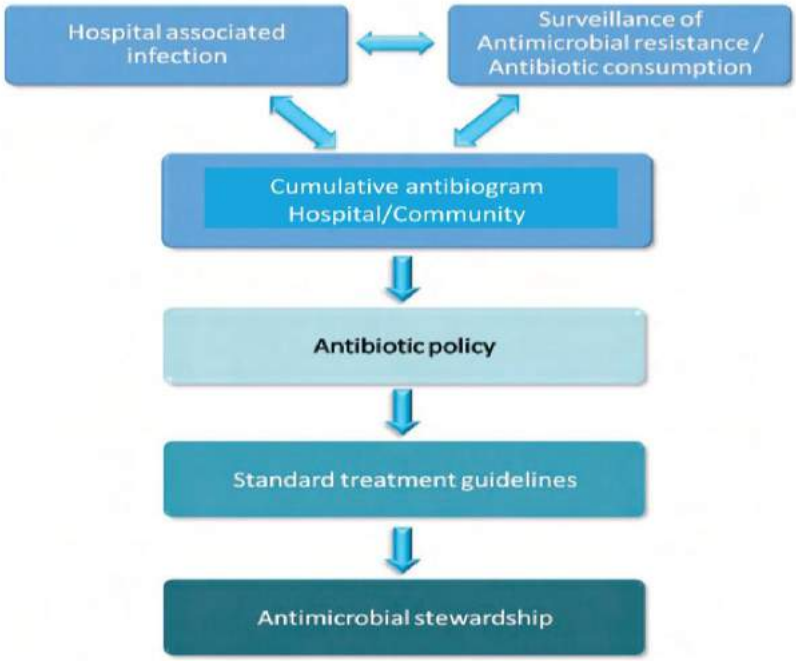

Fig 2: Flow chart of the process for writing antibiotic policy and guideline (35)

5.2.1. Human Resources

- Clinicians, clinical microbiologists, nurses, pharmacists, and IPC practitioners should form a team to write the antibiotic policy and guidelines
- At the primary health care facilities, where these health care workers are not available, "standing order" is used for antibiotic prescribing (usually of antibiotics in the Access group of WHO AWaRe classification). The "standing order" should be reviewed periodically based on an applicable antimicrobial susceptibility test report. The "standing order" may be available from the Primary Health Care Development Agency (PHCDA).
- The AMS Team may identify available evidence-based policies and guidelines (national or international) and adapt appropriately to fit local situations and write the first draft.
- The Team must have information on the local antibiotic formulary of the hospital or country and the antibiotics that are available locally.
- Local antibiotic susceptibility data should be provided by the clinical microbiology laboratory.
- A list of common infectious disease syndromes or infections based on the result of the Global-PPS should be provided.

5.2.2. Scope of Hospital Antibiotic Policy

Each hospital should design an antibiotic policy to govern prophylactic, empirical and targeted antibiotic therapy. In particular, there should be a policy on surgical antibiotic prophylaxis, antibiotic time-outs, use of the clinical laboratory to guide targeted therapy, and access to restricted or reserved antimicrobials. The guideline must align with the policy.

5.2.3. Content of Hospital Antibiotic Policy

- Contains a list of antibiotics for general use, reserved and restricted antibiotics
- Recommendations for principles of AMS
- Guidance for IV to oral switch
- AWaRe classification of antibiotics
- Policies for good antibiotic prescribing

5.2.4. Getting started with Guideline

- Choose your indications (common infections) in the health care facility based on the point prevalence survey of antibiotic use
- Look for available national and international guidelines, or guidelines from agencies, professional societies or ministries of health on antibiotic use, which may be adopted for your facility
- The AMS Team and stakeholders write the first draft of the guideline taking

into consideration the local antimicrobial susceptibility data. This can be done in each clinical department in a tertiary health care facility.
- This is disseminated to all stakeholders including resident doctors, medical officers, pharmacists among others for input.
- Based on the input from all stakeholders, a second draft is prepared.
- Do a pilot testing of the second draft in a section of the health care facility.
- Monitor and review the guideline based on the pilot testing.
- Write the final version of the guideline which will be approved by the antimicrobial stewardship committee.
- It is very important that antibiotic guideline is written by those who will use it so that they can take ownership and responsibility for implementation.

5.2.5. Contents of Guidelines

The following factors should be included in the contents of the guideline;

- Should be syndrome/disease based such as urinary tract infection (UTI), skin and soft tissue infection (SSTI), pneumonia
- Type of clinical setting: outpatient clinics, inpatient units, ICU setting
- When to switch from parenteral to oral route
- Clinical criteria for the diagnosis of infection/syndrome.
- Severity of illness
- Suggestion for diagnostic testing of disease condition(s)
- When to discharge
- Choice of antibiotics for empirical therapy, dose, route and duration of administration.

5.2.6. Selection of Antibiotics

In the selection of antibiotics, the following factors should be taken into consideration;

- Appropriate choice, dosage, route of administration, and duration of antibiotics
- Alternatives for allergy to first-line agents
- Adjusted dosage for patients with impaired liver or renal function including special needs of individual patient groups, for example, critically ill, pregnancy and obesity
- Severity of infection
- Site of infection
- Spectrum of antibiotic activity
- Pharmacokinetics/pharmacodynamics
- Adverse effects
- Potential to select resistance
- Cost of antibiotics, administration and monitoring microbiological causative microorganisms

5.2.7. Antibiotics Prophylaxis

- The duration of the prophylaxis should be specified
- Antibiotics dedicated for prophylaxis should not be used as first line or for empirical therapy except indicated by the results of AST.

5.3. Dissemination of the Antibiotic Policy and Guidelines

- Electronic copies sent to WhatsApp and e-mails of prescribers
- Print copies to be placed in the wards
- Pocket book copies that can be given to all prescribers
- Intranet within an electronic medical record
- Mobile applications

5.4. Audit of compliance to the Antibiotic Policy and Guidelines

- The periodic audit of antibiotic policy should focus on;
    - Documentation of the indication for antibiotic use
    - Stop/review date
    - IV-to-oral switch
- The periodic audit of antibiotic guideline should focus on compliance with current;
    - Clinical treatment guidelines
    - Guidelines for surgical prophylaxis
- Feedback of audit results to prescribers and health care facility management and other stakeholders is essential.

Note that:

- It is important that clinicians, pharmacists, nurses and other stakeholders are educated on the policy and guidelines and given an opportunity for feedback.
- Dissemination of the policy and guidelines should be given wide publicity
- It should be the responsibility of the AMS Committee/Team to assess and ensure the compliance of clinicians and other health care workers with their antibiotic policy and guidelines.
- Policy and guidelines are living docu-

ments, therefore, they should be reviewed at periodic intervals based on current medical knowledge, clinical practice and local circumstances.

Section 6: Engaging Stakeholders on Antimicrobial Resistance and Stewardship

Stakeholder engagement is critical to a successful ASP. Stakeholders become more informed about issues and are able to positively support such programmes. A good engagement clarifies the purpose of the activity; why it is important; clarifies the context; and sets a clear direction of action. Engagement is a means to an end.

For effective stakeholder engagement, the following factors should be taken into consideration;

- Identifying who the stakeholders are
- Understanding who has the most influence on the issue
- Sharing and delivering on expectations
- Showing an understanding of the stakeholders' concerns

AMS stakeholders include health care facility management, clinicians, pharmacists, nurses, IPC practitioners, clinical microbiologists, other relevant laboratory staff, patients, etc. The stakeholders should be engaged in the following areas; AMR awareness, appropriate antibiotic prescribing, IPC, diagnostic stewardship and surveillance.

6.1. Goals, Processes and Tools for AMS stakeholders' engagement

- **Goal 1**: To inform and inspire stakeholders

  This is usually a one-way communication to stakeholders to inform them about a health issue or policy matter. Tools/processes for this include handbills, fact sheets or meetings solely for information, for example 24-hour surgical prophylaxis compliance rates from Global-PPS results.

- **Goal 2**: To consult and listen to stakeholders

  Here, the stakeholders are given the opportunity to make input or suggestions to proposals but debates for or against these proposals are usually not done at this level. The tools/processes for this includes meetings, submissions, focal group discussions, surveys, for example determination of reasons for non-compliance with 24-hour surgical prophylaxis, and suggestions for improvement.

- **Goal 3:** To involve the stakeholders

  Stakeholders receive new information on the issues under consideration, deliberate on them and then come to an agreement and/or make recommendations to inform the decisions. The tools/processes for this include workshops, and committees, for example, form a group to review the information available on non-compliance with surgical prophylaxis, and proffer solutions.

- **Goal 4:** To collaborate with stakeholders

  Committees/sub-committees should be created from members who have some decision-making authority. The tools/processes to achieve this include participatory decision-making strategies, for example, a meeting of authorities involved in ensuring compliance with antibiotic guidelines at the highest level, such as meeting with the head of department and consultants in surgery

- **Goal 5:** To empower the stakeholders

  In this case, a committee is set up to undertake the responsibility entirely and tasked with addressing a public problem over an extended period of time. The tools/processes for this include a committee for implementation, for example, a committee to implement and monitor compliance with 24-hour surgical prophylaxis, such as the AMS Committee.

Note that each level of engagement deepens the involvement of the stakeholders.

6.2. Strategies for successful stakeholder engagement

- The reason or goal for engagement must be clear.
- Share the identified purpose with the stakeholders very early.
- Adopt the appropriate process; the type of engagement needs to be tailored to the purpose (see goals 1 to 5 above).
- Usually, stakeholders' engagement is most effective when it stretches to collaborative and empowerment levels.
- Stakeholders are not a uniform group, therefore, engagement needs to focus on a specific issue or topic and invo-

lves interaction with the groups most relevant to the issue.

6.3. Platforms for stakeholders engagement on AMS

- Hospital meetings/Grand rounds
- Focus group discussions
- Surveys
- Web-based engagements
- Print and electronic media
- Billboards, Posters, Handbills
- Social media
- Relevant committees and professional bodies/Associations
- Conferences and Workshops

6.4. Categories of stakeholders

The knowledge and attitude of stakeholders on particular issues vary, and must be considered during engagements (36).

- **The unaware:** These are not aware of the issues (for example AMR, AMS) and their potential impact such as high mortality and morbidity
- **The resistant:** These are aware of the issues and potential impacts, but are resistant to change
- **The neutral:** These are aware of the issues, but are neither supportive nor are resistant to change
- **The supportive:** These are aware of the issues and potential impacts, and are supportive of change
- **The leading:** These are aware of the issues and potential impacts, and are actively engaged in ensuring the programme is a success

The overarching target is to make all stakeholders become supportive towards the programme.

Section 7: Education and Training

Training and education are paramount to the successful implementation of interventions in ASPs (37). However, it is most effective when paired with interventions and measurement of outcomes (29). The health care facility should provide training to all staff on AMS, during new staff orientation programmes and provide continuous in-service training or continuous professional development on AMS and IPC (38).

7.1. Training objectives should be to:

- Create awareness of AMR, AMS and their relationship
- Provide knowledge on the drivers and impact of AMR
- Impart detailed knowledge of ASPs
- Promote behavioural change in antimicrobial prescribing, use, procurement, dispensing and disposal
- Educate new or rotating staff and students
- Emphasise and promote the need for appropriate use of the clinical microbiology laboratory
- Update and reinforce previous knowledge
- Encourage continuous quality improvement

7.2. Targets of Education & Training

Activities should target various audiences using behavioural change communication strategies. These targets should include:

7.2.1. Antimicrobial Stewardship Committee and Team(s)

The members of the AMS Committee (AMSC) should be adequately trained on AMS and the ASP including principles/actions required for its establishment and implementation, antimicrobial policy and guidelines, and IPC. This should be done at the inception of the Committee and reinforced periodically. Training should be practical and specific to the roles of each member. Award of certificates of participation would be a motivation and an added advantage.

Administrative heads of health care institutions should support AMSC and AMS Team(s) (AMSTs) by facilitating opportunities for training and retraining, to reinforce previous training as well as updating their knowledge. They should also provide funding and foster collaboration with partners, nationally and globally to support their ASPs (29). Resources on practical guidance for implementing an ASP should be made available and accessible when required.

Hospital AMS campaigns should be publicised. This is necessary to gain support for successful interventions in identified priority areas. Critical stakeholders such as clinicians and other health care providers should be educated on the proposed activities around the priority area. AMS champions in the specialty(ies) in focus should lead publicity efforts in their units. Heads of health care facilities and key opinion leaders may be made ambassadors to successfully drive the programme.

7.2.2. Clinicians, Pharmacists, Nurses, and other health care staff

This should be an ongoing process with trainings repeated as much as feasible.

Education should cover the fundamentals of AMR, AMS, the importance of proper and optimised diagnosis, and promote adherence to antimicrobial policy and guidelines. Structured training relevant to specific AMS interventions is essential.

Opportunities for training include orientation programmes for new staff, grand rounds, clinical meetings and undergraduate medical education by including it in the curriculum. information, education and communication (IEC) materials (posters, fliers, banners, text messages, social media posts) shared physically, via telephone and on social media, could be used to promote ASP activities. Telemedicine could provide AMS support for prescribers such as mobile Apps for AMS and antimicrobial guidelines (38). A health care worker guide has been published by the Public Health England and the WHO with details on the training of various health care workers (34).

7.2.3. Patients and Caregivers

These stakeholders should be educated on the inappropriate use of antimicrobials, negative consequences of inappropriate antimicrobials use, including sharing, use of leftovers, inappropriate disposal and demand for antibiotics. Fora such as the antenatal clinic (ANC), immunisation clinics and waiting areas in consulting clinics should be utilised to drive the health education.

They should know what antibiotics they are receiving, and for what reason(s), and about their adverse effects (signs and symptoms to identify these) including those that may occur after discharge or stopping antibiotics. They should be encouraged to be antimicrobial guardians. Engaging patients and caregivers in the development and review of educational (IEC) materials is beneficial (29).

7.2.4. Community

This includes teachers and educators, journalists and communication experts, social media influencers, religious leaders, and the general community including children and young persons. To support AMS at the community level, they should be educated regularly on antimicrobial resistance, ills of self-medication and the responsible use of antibiotics. This can be routinely done by trained public health, ASP ambassadors and advocates, and family medicine practitioners (39).

7.2.5. Advocacy and community campaigns

Regular engagement and awareness creation campaigns on the threat of Antimicrobial resistant infections and measures to contain them, should be carried out by AMS practitioners using data generated locally. Highly visible and regular national advocacy activities, among policy-makers and the general public (such as during World Antimicrobial Awareness Week) are beneficial, to raise the political and public profile of AMS and to get their "buy-in" to AMS programmes (39).

Section 8: Monitoring and Evaluation

Monitoring and evaluation (M & E) are critical to identifying opportunities for improvement and the overall impact of intervention measures. M & E involves the evaluation of structures, processes and outcomes of ASPs.

8.1. Monitoring

Monitoring is a systematic and routine collection of data to enable implementers to determine whether the objectives of the programme are being achieved. The attainments are compared to set targets during evaluation and actions taken to address gaps or deficits. Data acquired through monitoring is used for evaluation.

8.2. Evaluation

Evaluation involves assessment of a programme as systematically and objectively as possible, at defined time points. It appraises data and information that inform strategic decisions and helps to draw conclusions about the relevance, effectiveness, efficiency, impact, and sustainability of the intervention. It is an integral concept in every project or programme design.

8.3. Approach to Monitoring and Evaluation in AMS

For convenience and ease of understanding, monitoring and evaluation in AMS could be grouped into structure, processes, and outcomes.

8.3.1. Structure:

This should cover aspects that include the composition, terms of reference and governance structure of the AMS Committee and Team(s). Membership and composition of the Committee/Teams are as highlighted in Section 3.4 of this document. The CLIMIDSON AMS checklist (www.climidson.org.ng) can be used as a tool for measurement.

8.3.2. Processes:

The processes in this context include quality indicators in the application and implementation of AMS which can best be evaluated

using the Global-PPS and audit of specific antimicrobials. Some of the process measures/ indicators based on Global-PPS include documented indication for antibiotic use, stop/ review date, compliance with current clinical treatment guidelines, de-escalation, and IV-to-oral switch. Others include prevalence of antibiotic consumption measured as defined daily doses (DDD) or day of therapy (DOT), prevalence of antimicrobial prescribing, and surgical antibiotic prophylaxis (SAP) not beyond 24 hours.

8.3.3. Outcomes:

Outcomes of AMS represent the intended benefits which include AMR rates, mortality rates and length of hospital stay. Outcome indicators should be defined at the planning stage of the ASP and monitored while implementing the programme.

Section 9: Feedback of Data from Surveillance and M&E

This entails the distribution of results and outcome of AMS interventions and surveillance activities to those who need to know and act. This is essentially communicating the outcome to relevant stakeholders, including but not limited to hospital management and prescribers. This can be done using appropriate media and platforms such as presentations at clinical meetings/grand rounds, advocacy, fliers, posters and scientific publications.

During dissemination, opportunities should be created for responses from the stakeholders to the feedbacks. Such responses can then be used to improve the AMS action plan. These include feedback to prescribers, AMS Committee and Teams, and health care facility management on:

- Global-PPS data
- Antibiotic consumption data
- AMS interventions and actions
- AMR surveillance and antibiogram

Section 10: Clinical Microbiology Laboratory Support and Diagnostic Stewardship

10.1. Strengthening Laboratory Capacity for Hospital ASPs

The clinical microbiology laboratory plays a key role in ensuring the success of the antimicrobial stewardship programme. In order to provide quality services and reliable data, clinical laboratories should ensure the followings:

10.1.1. Provide guidance to laboratory users

The laboratory should provide documents (laboratory handbook, periodic advisories) with details of the policies and operations of the laboratory including; hours of operation, proper collection and transport of laboratory specimens, appropriate use of laboratory services and other relevant information (29). These documents should be made accessible and available to the laboratory users. It should also provide interpretation of laboratory results and reports.

10.1.2. Provide quality laboratory services including Antibiotic Susceptibility Testing (AST)

The laboratory should perform the right test requested at a minimal turn-around-time (TAT), release laboratory reports to health care providers in a timely manner to guide physician decisions on treatment and optimise communication of critical test result values and alert systems (40,41). With respect to AST, the laboratory should:

- Ensure quality-assured AST data are available
- Adhere to standardised protocols with appropriate quality controls
- Be involved in proficiency testing (PT)
- Test supplementary antimicrobial agents in the event of resistance
- Promptly report unusual patterns of AMR
- Provide advice to the physician on therapy for such patients.
- Update methods for susceptibility testing periodically

The use of automated or semi-automated testing systems is encouraged where possible, especially when dealing with severe life-threatening infections. The use of single antibiotic disc for AST offers the staff the opportunity to choose specific antibiotics for testing against specific organisms.

10.1.3. Promote Diagnostic Stewardship

Diagnostic stewardship (DS) is the coordinated guidance and interventions to improve the appropriate use of microbiological diagnostics to guide therapeutic decisions (42). It refers to the appropriate use of laboratory testing, including use of biomarkers, to guide patient management, in order to optimise clinical outcomes and limit the unnecessary use of antibiotics and spread of AMR.

This requires synergy between clinical laboratories and physicians so that appropriate tests are requested, and laboratory reports are translated into appropriate antimicrobial management (43). DS aims to promote appropriate and timely test request/ordering, specimen collection, specimen processing and reporting. The process of implementing DS is summarised in Fig 3 (44).

10.1.4. Continuous laboratory quality improvement

The laboratory should upgrade diagnostic capacity to:

- Perform standardised tests, which include microscopy, culture, identification (ID) and AST.
- Use rapid diagnostic technologies for targeted critical specimen types and detection of AMR
- Test for biomarkers of infection including non-culture-based fungal markers.
- Promote appropriate use of point-of-care microbiological tests, when available (41).

10.2. Antimicrobial resistance surveillance

Antimicrobial resistance surveillance involves tracking changes in microbial populations for early detection of resistant strains of clinical and public health importance, and reporting on trends in resistance on a periodic basis. Clinical laboratories should collaborate with the institution's AMS committee, using information from the analysis of AST, to determine priority pathogens, syndromes, patient populations, antimicrobials and provide target for stewardship interventions. Thus, AMR surveillance data are needed to;

- inform clinical therapy decisions
- guide policy recommendations and treatment guidelines development
- assess the impact of ASP interventions to contain AMR

At the facility level, regular analyses of AMR data should be provided to groups with responsibility for antimicrobial guidelines (i. e. AMSC/AMST, DTC) to inform empirical therapy recommendations and formulary management.

A specification for health care facility cumulative antibiogram is an essential step towards achieving detailed, accurate and efficient AMR surveillance. To achieve this, health care facilities should ensure that;

- A standard technical specification of a facility cumulative antibiogram is developed using the appropriate CLSI document (45)
- There is ongoing maintenance and revision of the cumulative antibiogram specification.
- Clinical microbiology laboratory should report signal resistances from antibiogram data for the health care facility's ASP annually.

10.3. Antibiogram

An antibiogram is an overall profile of antimicrobial susceptibility testing results of a specific microorganism to a battery of antimicrobial drugs within a defined period (45). This is produced periodically, usually annually, to provide information on common pathogens

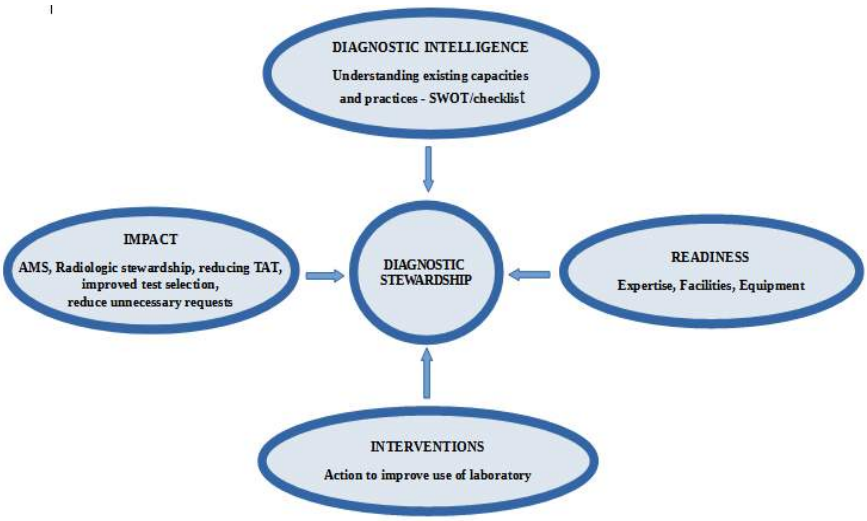

Fig 3: Implementation of Diagnostic Stewardship (adapted from 44)

and their antibiotic susceptibility patterns to guide the choice of empirical antibiotics.

Antibiogram should be derived from records of culture and antibiotic susceptibility testing carried out during the period under consideration.

10.3.1. Uses of an antibiogram

- Helps in guiding the clinicians and AMS team in the selection of best empirical antimicrobials
  treatment in the event of pending microbiology culture and susceptibility results. It should be the basis for facility and national guidelines development.
- Useful tools for detecting and monitoring trends in AMR, which can then be investigated.

10.3.2. Specifications for antibiogram

- Cumulative antibiograms should be produced differently, for urine, non-urine and blood culture isolates.
- For statistical significance, there should be at least 30 isolates at the species level. However, if this is not possible, the isolates can be grouped by genus or family.
- In smaller laboratories, cumulative antibiograms may be produced for all isolates combined irrespective of specimen type but should be categorized into Gram-negative and Gram-positive bacteria (Tables 2a and 2b).
- When the antibiotic data from less than 30 isolates is presented, it is recommended that a footnote should state that the results may not have attained a statistically significant measure of susceptibility in that microbial population
- Each cumulative antibiogram should consist of data for one calendar year and be published early in the following year.
- Only the antibiotic susceptibility data from the first isolate of a bacterial species from an individual patient should be included to eliminate duplicates. If the same bacteria species is isolated from urine, a non-urine site or blood from an individual, then the susceptibility data from the first isolate from each site should be included in their respective antibiogram.
- Only validated antibiotic susceptibility test results from clinical isolates submitted for diagnostic purposes should be included.
- For each genus, species or other groupings, the number of isolates (denominator) used in determining the percentage should be noted on the antibiogram report.
- Limitations of the antibiogram should be listed in the footnote.

10.4. Signal Resistance

Certain combinations of microorganisms and antibiotic resistance should be reported in a specialised antibiogram to draw attention to multi-drug resistant organisms (MDROs) and resistant mechanisms (Tables 3a and 3b). With automated ID and AST methods, these are more readily detectable and characterised. The frequency of these should be reported including zero occurrences. Signal Resistances may include:

- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus* (VISA, VRSA). The method used for identifying VRSAs should be reported.
- Vancomycin-resistant Enterococci
- *Enterobacterales* resistant to third or later generation cephalosporins. Where the genetic mechanism for this resistance has been determined such as with ESBL, the method should be reported
- Carbapenem-resistant *Enterobacterales* (CRE) and other plasmid mediated carbapenemase producing Gram-negative bacteria such as *Acinetobacter* spp and *Pseudomonas aeruginosa.*
- *Streptococcus pneumoniae* with a penicillin MIC ≥ 0.06 µg/ml. These should be categorised as intermediate (MIC 0.12 – 1µg/ml) and resistant (MIC > 2 µg/ml) referring in the commentary to the fact that breakpoints for meningitis differ.

Section 11: Role of Information and Communication Technology (ICT) in the AMS Programme

The following are areas where ICT can be of benefit to AMS (46,47);

- Electronic medical records/hospital information system. This can be used for interventions such as audit, and for monitoring and evaluation.
- Database on procurement and ward dispensing at the facility pharmacy level. This can be used in calculating antimicrobial consumption and establish trend over time.
- Database of AMR surveillance in different units/departments

Table 2: Template for presenting cumulative antibiogram [Health care facility Logo]

(a): Cumulative antibiogram for all hospital isolates (Gram-Negative Bacteria) (Jan – December)

Bacterial Pathogen	No of Isolates Tested	Percent Susceptible																			
		Ampicillin	Ampicillin/ Sulbactam	Piperacillin/ Tazobactam	Cefazolin	Cefepime	Cefoxitin	Ceftazidime	Ceftriaxone	Cefuroxime	Aztreonam	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Levofloxacin	Trimethoprim/ Sulfa	Nitrofurantoin*	Tetracycline
Escherichia coli																					
Klebsiella pneumoniae																					
Proteus mirabilis																					
Pseudomonas aeruginosa																					

Antibiogram with <30 isolates are of questionable statistical significance; interpret data with caution
* = Nitrofurantoin is reported for urine sources only; Antibiogram with <30 isolates are of questionable statistical significance; interpret data with caution
NB: The antibiogram should report antibiotic susceptibilities for the antibiotics in actual clinical use, not the susceptibility to any surrogate antibiotic used in the laboratory e. g. for a laboratory using CLSI method, the antibiogram should report as percentage susceptible to flucloxacillin and not percentage susceptible to cefoxitin for Staphylococcus aureus.
If the 'breakpoint' for any antimicrobial-organism pair has changed since the last publication of a cumulative antibiogram for an institution, then the date the change was implemented should be indicated in a footnote to the table.

(b): Cumulative antibiogram for all hospital isolates (Gram-Positive Bacteria) (Jan – December)

Pathogen	No of Isolates Tested	Percent Susceptible													
		Penicillin	Ampicillin	Oxacillin[1]	Gentamicin	Ciprofloxacin[2]	Levofloxacin[2]	Moxifloxacin	Trimethoprim/ Sulfa	Clindamycin[3]	Daptomycin[4]	Nitrofurantoin[5]	Linezolid	Vancomycin	Tetracycline[2]
Staphylococcus aureus (MSSA)															
Staphylococcus aureus (MRSA)															
Enterococcus faecalis															
Enterococcus faecium															
Viridian streptococci															
Coagulase negative staphylococcus[6]															

1. Oxacillin predicts susceptibility to most cephalosporins, carbapenems, and beta-lactam/beta-lactamase inhibitors; 2. For *E faecalis*: ciprofloxacin, levofloxacin, tetracycline is reported for urine sources only
3. For *Staphylococcus* species: clindamycin is reported for non-urine sources only; 4. For *Staphylococcus* species: daptomycin is reported for non-respiratory sources only; 5. Nitrofurantoin is reported for urine sources only
6. Only clinically significant isolate should be reported

Table 3: Template for signal resistance reporting [Health care facility logo]

(a): Multidrug resistant bacteria isolated from non-urine (January – December)

Extended Spectrum Beta-Lactamase Producing Enterobacterales (ESBL)			
Bacteria	**Total No of isolates Tested**	**No ESBL Positive**	**% ESBL positive**
Escherichia coli			
Klebsiella spp			
Enterobacter spp			
Citrobacter spp			
Carbapenem-Resistant Enterobacterales (CRE)			
Bacteria	**Total No of Isolates Tested**	**No CRE Positive**	**% CRE positive**
Escherichia coli			
Klebsiella spp			
Enterobacter spp			
Citrobacter spp			
Vancomycin Resistant Enterococci (VRE)			
Bacteria	**Type**	**No VRE Positive**	**% VRE positive**
Enterococcus spp total			
	Enterococcus faecalis		
	Enterococcus faecium		
Methicillin-Resistant *Staphylococcus aureus* (MRSA)			
Bacteria	**Type**	**No MRSA positive**	**% MRSA positive**
S. aureus total			
	S. aureus (non-multi-resistant)		
	S. aureus (multi-resistant hospital-associated)		
Streptococcus pneumoniae* Penicillin Susceptibility			
***S. pneumoniae* (Oral Penicillin V Breakpoint)**	**MIC category**	**No of Isolate**	**% Isolate**
	Sensitive (≤ 0.06 µg/ml)		
	Intermediate (0.12 - 1 µg/ml)		
	Resistant (≥ 2 µg/ml)		
*Breakpoint for non-meningitis isolate IV treatment (R ≥ 8 µg/ml), meningitis isolate IV treatment (R ≥ 2 µg/ml)			
Viridians Streptococcus Group Penicillin Susceptibility			
Viridian streptococci	**MIC category**	**No of Isolate**	**% Isolate**
	Sensitive (≤ 0.12 µg/ml)		
	Intermediate (0.25 – 2 µg/ml)		
	Resistant (≥ 4 µg/ml)		

(b): Multidrug resistant bacteria isolated from urine (January – December)

Extended Spectrum Beta-Lactamase Producing Enterobacterales (ESBL)			
Bacteria	**Total No of isolates Tested**	**No ESBL Positive**	**% ESBL Positive**
Escherichia coli			
Klebsiella spp			
Enterobacter spp			
Citrobacter spp			
Carbapenem-Resistant Enterobacterales (CRE)			
Bacteria	**Total No of Isolates Tested**	**No CRE Positive**	**% CRE Positive**
Enterobacterales (total non-duplicates reported)			
Vancomycin Resistant Enterococci (VRE)			
Organism	**Types**	**No VRE Positive**	**% VRE Positive**
Enterococcus spp total			
	Enterococcus faecalis		
	Enterococcus faecium		
Methicillin-Resistant *Staphylococcus aureus* (MRSA)			
Organism	**Types**	**No MRSA Positive**	**% MRSA Positive**
S. aureus total			
	S. aureus (non-multi-resistant)		
	S. aureus (multi-resistant hospital-associated)		

- Alerts on specific antibiotic use can be generated and used to check the AMS process especially when restricted antibiotics are prescribed
- Time-sensitive automatic stop orders
- Electronic guidelines (via electronic mailings to prescribers, intranet)
- Adapted for mobile applications for antibiotic guidelines and other relevant documents.
- Point-of-care access to microbiological results from all units
- Clinical decision-support system

Section 12: Outpatient Antimicrobial Stewardship (OPAS)

Although the principles of AMS in the in-patient and out-patient sections of the health care facility are essentially the same, the approach to implementation may vary depending on the facility and the resources available to it. This manual is focused mainly on in-patients, but it was considered necessary to accommodate some principles and recommendations for out-patient AMS. The recommendations here may therefore apply to stand-alone clinics and casualties or those located in secondary or tertiary hospitals.

The outpatient AMS may be based on the 4 core elements of the CDC outpatient stewardship checklist for both clinician and facility (48).

12.1. Commitment

12.1.1. Leadership:

Health care facility leadership will create a multidisciplinary AMS team to collaborate on AMS activities, including representatives from clinicians, nursing, pharmacy, information technology, electronic health records, infection prevention, and quality improvement. AMS activities include;

- Regular meetings (monthly or quarterly)
- Provision of AMS guidelines, protocols, and defined prescribing standards and recommendations
- Tracking and reporting at least one aspect of antibiotic prescribing
- Making an AMS action plan and reviewing annually as needed
- Receiving feedback from clinicians about current AMS interventions.
- Reviewing the AMS plan document annually and revising as needed.
- Communicating prescribing standards to staff and providers.

The same facility AMS set up for in-patient programme can also serve the out-patient section. There is no need for a dual AMS committee

12.1.2. Accountability:

Health care facility leadership will identify one physician leader to be responsible for AMS oversight and promotion. He/she will lead the AMS team. AMS responsibilities will be included in job description or performance review criteria.

12.1.3. A written commitment statement in support of AMS will be posted in the facility, visible to patients, families, and all staff.

12.1.4. AMS team will regularly communicate with all clinic staff to manage patients' expectations.

12.2. Action for Policy and Practice

These are intended to support appropriate prescribing practices by individual prescribers and to inform all staff about the importance of AMS. The actions should include;

- Evidence-based diagnostic criteria and Antibiotic policy and treatment guideline
- Policy to document the indication for antimicrobial prescription in the patient's case notes including the dose, duration, route of administration and frequency.
- Delayed prescribing and watchful waiting. This involves the supply of antibiotic prescription to a patient with clear instructions about when to commence the treatment in relation to their symptoms. It is useful for conditions that usually resolve without treatment but which can benefit from antibiotics if the condition does not improve.
    - Patient communication required when initiating a watchful waiting period include; diagnosis, suggestions for symptom relief (including any non-antibiotic medications), and instructions for follow-up.
- Provide communication skills training for clinicians through;
    - Annual communication skills training to enhance their ability to address patient concerns about prognosis, benefits and harms of antibiotics and management of self-limiting conditions,
    - To address clinician concerns about managing patient expectations for antibiotics.

12.3. Tracking and Reporting

- Track and report antibiotic prescribing for one or more high-priority conditions such as upper respiratory tract infection, acute bronchitis, sinusitis, pharyngitis and acute watery diarrhoea.
- Track and report the percentage of all visits leading to antibiotic prescriptions. This can be done as a point prevalence survey using the out-patient Global-PPS.
- Assess documentation of indication for antibiotic prescription in the patients' case note.
- List of staff who have completed communications skills training.
- Feedback on tracking of antibiotic prescribing for high priority conditions
- Monthly or quarterly AMS reports to the health care workers and health care facility leadership
- Antibiogram

12.4. Education and Expertise

- Use effective communications strategies to educate patients and families about when antibiotics are and are not needed.
- Educate patients and families about the potential harms of inappropriate antibiotic treatment
- Provide patient education materials
- Provide continuing education activities for clinicians
    - Annual in-service training
    - Induction or orientation training for new staff

Section 13: Mentorship in AMS

Antimicrobial stewardship mentorship will contribute to capacity building and support towards the implementation of the ASP. The aim of the AMS mentorship is to improve knowledge, performance and skills of the AMSC and AMSTs in carrying out an effective ASP in various institutions.

The mentorship programme requires clear goals and objectives, as well as an agreement on the method and frequency of communication. Mentorship could be between individuals or between institutions/organisations or institutions/organizations and individuals. The mentor will be an expert in antimicrobial resistance and stewardship or an institution that is advanced in AMR/AMS practices (49).

The roles of a mentor are to motivate and;

- Advice and support the facility to develop multidisciplinary AMSC/AMST
- Increase the knowledge of the facility AMSC/AMST on AMR and AMS and develop action plan.
- Train the facility AMSC/AMST on the principles of rational AMU and AMS
- Train facility AMSC/AMST on how to monitor and evaluate AMS interventions using Global-PPS and other methods
- Assist facility AMSC/AMST in developing AMS policies and guidelines including antibiotic guidelines
- Support the health care facility in strengthening diagnostic stewardship or assisting in the analysis of microbiological investigations as the case maybe.
- Advice facility AMSC/AMST on how to carry out communication and awareness programmes amongst staff
- Advice and guide facility AMSC/AMST on grant applications in order to promote researches on AMR and AMS

It is also essential to establish KPIs that should be used to measure the impact of the mentorship programme. Collection of both qualitative and quantitative data is important in the assessment of the impact of the mentorship. The key indicators that should be focused on are as follows;

- Engagement indicator: This will cover data collection on mentoring sessions, mentoring hours and actions taken
- Process indicator: This will include collecting data on the number of goals established and achieved, and personal satisfaction scores

AMS mentorship initiatives can be conducted following a step-wise pattern;

- Application by mentee institution to AMS mentor, for example CLIMIDSON, NASWOG
- Complete a pre-visit survey (for example with CLIMIDSON AMS checklist) including information about the facility's current ASP. This information will be critical to a successful onsite visit.
- Mentoring onsite visit: The mentor will work with the AMS Team leader and other members of the AMS Team by phone in advance of and following the onsite visit.

- The onsite visit will feature an in-depth evaluation of the facility's AMS programme, practices, challenges and opportunities for improvement, and proposed activities to enhance patient care. Presentations can be made during the visit and specific activities during the visit will vary according to mentee institution goals and needs but may include meeting with the AMS Team and other teams (such as IPC and quality improvement teams), as well as reviewing protocols, policies and procedures.
- Following the site visits, the mentor will provide a customised report of proposed interventions to improve the facility ASP.
- Report outcomes: Following a visit, the facility (mentee) is expected to implement AMS improvement project and report key outcomes approximately 6 months after the onsite visit.

Section 14: System Building and Sustainability Plan in AMS

The sustainability plan in AMS for health care facility requires that important factors that can influence programme implementation and sustainability be identified (50). It is important to recognise ASP as a behaviour change programme, and that changing prescribing habit cannot occur over-night (51). Implementing and sustaining ASPs in health care facilities will be facilitated by;

- Having an ASP sustainability plan
- Continuous support of the management in terms of human, material and financial resources.
- Facility management need to recognise AMS as a quality improvement programme and include it in their annual performance indicators.
- Having leaders who are passionate and knowledgeable on the issues of AMR/AMS.
- Regular and periodic performance of Global-PPS as integral part of M & E to measure progress and success.
- Prioritising interventions that are easier to perform such as supplemental strategies like education, guideline development, IV-to-PO switch, de-escalation, dose optimisation, and microbiology strategies. Start small and then scale up the interventions.
- Ensuring ongoing education training with feedback of relevant personnel.
- Should be all inclusive: All stakeholders should be involved.
- Provision should be made for both internal and external mentorship

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References:

1. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. PT. 2015; 40 (4): 277-283.
2. World Health Organization. 2021. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
3. O'Neill, J. Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. 2014.
4. Prestinaci, F., Pezzotti, P., and Pantosti, A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015; 109 (7): 309-318. doi:10.1179/2047773215Y.0000000030
5. World Health Organization. Global Action Plan on Antimicrobial Resistance. World Health Organization; 2015.
6. Gitaka, J., Kamita, M., Mureithi, D., et al. Combating resistance using guidelines and enhanced stewardship in Kenya: a protocol for an implementation science approach. BMJ Open. 2020; 10: e030823. doi:10.1136/ bmjopen-2019-030823
7. Murray, C. J. L., Ikuta, K. S., Sharara, F, et al. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022; 399 (10325): 629-655. doi:10.1016/S0140-6736(21)02724-0
8. Tacconelli E, Górska A, De Angelis G., et al. Estimating the association between antibiotic exposure and colonization with extended-spectrum β-lactamase-producing Gram-negative bacteria using machine learning methods: a multicentre, prospective cohort study. Clin Microbiol Infect. 2020; 26 (1): 87-94. doi:10.1016/j.cmi.2019.05.013
9. Minarini, L. A. D. R., Andrade, L. Nd., De Greg-

orio, E., et al. Editorial: Antimicrobial Resistance as a Global Public Health Problem: How Can We Address It. Front Publ Health. 2020; 8: 612844. doi:10.3389/fpubh.2020.612844
10. Dadgostar, P. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 2019; 12: 3903-3910. doi: 10.2147/IDR.S234610
11. Bernabé, K. J., Langendorf, C., Ford, N., Ronat, J., and Murphy, R. A. Antimicrobial resistance in West Africa: a systematic review and meta-analysis. Int J Antimicrob Agents. 2017; 50 (5): 629-663. https://doi.org/10.1016/j.ijantimicag.2017.07.002
12. 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
13. Ayukekbong, J. A., Ntemgwa, M., and Atabe, A. N. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control. 2017; 6: 47. https://doi.org/10.1186/s13756-017-0208-x
14. Fleming, A. Penicillin Nobel Lecture, December 11, 1945
15. World Health Organization. GLASS manual on the management of antimicrobial consumption data. Geneva: World Health Organization; 2020.
16. Hijazi, K., Joshi, C., and Gould, I. M. Challenges and opportunities for antimicrobial stewardship in resource-rich and resource-limited countries. Exp Rev Anti-infect Ther. 2019; 17 (8): 621-634 https://doi.org/10.1080/14787210.2019.1640602
17. 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 (1): 42-46. doi: 10.4103/atp.atp_38_17
18. Ekuma, A. E., Ijezie, E., Akpabio, T., and Onwuezobe, I. A. Survey of antimicrobial consumption in a university teaching hospital in Southern Nigeria. Ann Trop Pathol. 2019; 10: 48-51. doi: 10.4103/atp.atp_50_18
19. Abubakar, U. Antibiotic use among hospitalized patients in northern Nigeria: a multi-center point-prevalence survey. BMC Infect Dis. 2020; 20: 86. https://doi.org/10.1186/s12879-020-4815-4
20. Aboderin, A. O., Adeyemo, A. T., Olayinka, A. A., et al. Antimicrobial use among hospitalized patients: A multi-center, point prevalence survey across public healthcare facilities, Osun State, Nigeria. Germs. 2021; 11 (4): 523-535. doi: 10.18683/germs.2021.1287
21. Nnadozie, U. U., Umeokonkwo, C. D., Maduba, C. C., et al. Patterns of antimicrobial use in a specialized surgical hospital in Southeast Nigeria: Need for a standardized protocol of antimicrobial use in the tropics. Niger J Med. 2021; 30: 187-191. doi: 10.4103/NJM.NJM_225_20
22. McGowan, J. E Jr., and Gerding, D. N. Does antibiotic restriction prevent resistance? New Horizon 1996: 4 (3): 370–376.
23. Shrestha, J., Zahra, F., and Cannady Jr, P. Antimicrobial Stewardship. StatPearls Publishing; 2022.
24. Babatola, A. O., Fadare, J. O., Olatunya, O. S., et al. Addressing antimicrobial resistance in Nigerian hospitals: exploring physicians prescribing behavior, knowledge, and perception of antimicrobial resistance and stewardship programs. Expert Rev Anti Infect Ther. 2021; 19 (4): 537-546.doi:10.1080/14787210.2021.1829474
25. Ogoina, D., Mahmood, D., Oyeyemi, A. S., et al. A national survey of hospital readiness during the COVID-19 pandemic in Nigeria. PLoS One. 2021; 16 (9): e0257567. https://doi.org/10.1371/journal.pone.0257567
26. Iregbu, K. C., Osuagwu, C. S., Umeokonkwo, C. D., et al. Underutilization of the Clinical Microbiology Laboratory by Physicians in Nigeria. Afr J Clin Exper Microbiol. 2020; 21 (1): 53-59. doi: 10.4314/ajcem.v21i1.7
27. Nwajiobi-Princewill, P. I., Medugu, N., Gobel, M., et al. Using longitudinal antibiotic point prevalence survey (PPS) to drive antimicrobial stewardship programmes in a Nigerian tertiary hospital. Afr J Clin Exper Microbiol. 2021; 22(2): 284-289. https://dx.doi.org/10.4314/ajcem.v22i2.22
28. Daily Sun Newspaper, August 29, 2018
29. Centers for Disease Control and Prevention. Core Elements of Hospital Antibiotic Stewardship Programs. Atlanta, GA: US Department of Health and Human Services, CDC; 2019. https://www.cdc.gov/antibiotic-use/core-elements/hospital.html
30. World Health Organization. Antimicrobial stewardship programmes in health-care facilities in low- and middle-income countries. A practical toolkit. Geneva: World Health Organization; 2019.
31. World Health Organization. The 2019 WHO AWaRe Classification of Antibiotics for Evaluation and Monitoring of Use. Geneva, Switzerland. World Health Organization. 2019.
32. Butler, C. C., Simpson, S. A., Dunstan, F., et al. Effectiveness of multifaceted educational programme to reduce antibiotic dispensing in primary care: practice based randomised controlled trial. BMJ. 2012; 344: d8173. doi: 10.1136/bmj.d8173
33. Oduyebo, O. O., Iregbu, K., Nwajiobi-Princewill, P. I., Versporten, A., Temiye, E., Fajolu, I., and Goossens, H. The Global Point Prevalence Survey of Antimicrobial Consumption and Resistance (Global-PPS): 2017 Longitudinal Results of Antimicrobial Prescribing in Nigerian Hospitals. A paper presented at the 13th Annual Scientific Conference and All Fellows Meeting (ASCAF) of the National Postgraduate Medical College of Nigeria (NPMCN) Owerri, August 2018.
34. World Health Organization. Health workers' education and training on antimicrobial resistance: Curricula guide, 2019.
35. World Health Organization. Step-by-step approach for development and implementation of hospital antibiotic policy and standard treatment guidelines. World Health Organization, Regional Office for South-East Asia, New Delhi-110 002, India, 2011
36. Stowe, L. Stakeholder Engagement: How to Measure & Report on Relationships in Government Affairs. Fiscal Note (Online) May 2022. https://fiscalnote.com/blog/measure-stakeholder-engagement-government-affairs
37. Centers for Disease Control and Prevention. The core elements of human antibiotic stewardship programs in resource-limited settings: national and hospital levels. Atlanta: US Department of Health and Human Services. 2018. https://www.cdc.gov/antibiotic-use/healthcare/pdfs/stewardship-resource-limited-508.pdf.
38. Barlam, T. F. The state of antibiotic stewardship programs in 2021: The perspective of an experienced steward. Antimicrob Steward Health Epidemiol. 2021; 1 (1): e20. doi: 10.1017/ash.2021.180
39. Mathew, P., Sivaraman, S., and Chandy, S. Communication strategies for improving public awareness on appropriate antibiotic use: Bridging a vital gap for action on antibiotic resistance. J Family Med Prim Care. 2019; 8 (6): 1867-1871. doi:10.4103/jfmpc.jfmpc_263_19
40. Morency-Potvin, P., Schwartz, D. N., and Weinstein, R. A. Antimicrobial stewardship: how the microbiology laboratory can right the ship. Clin Microbiol Rev. 2017; 30 (1): 381-407.
41. Barlam, T. F., Cosgrove, S. E., Abbo, L. M., et al. Implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. Clin Infect Dis. 2016; 62 (10): e51-e77.
42. World Health Organisation. Diagnostic steward-

ship: A guide to implementation in antimicrobial resistance surveillance sites. WHO, Geneva, Switzerland. 2016. WHO/DGO/AMR/2016.3. https://apps.who.int/iris/bitstream/handle/10665/251553/WHO-DGO-AMR-2016.3-eng.pdf

43. Patel, R., and Fang, F. C, Diagnostic Stewardship: Opportunity for a Laboratory–Infectious Diseases Partnership, Clin Infect Dis. 2018; 67 (5): 799–801. https://doi.org/10.1093/cid/ciy077
44. Prinzi, A. Diagnostic Stewardship Interventions That Make a Difference. ASM, Aug 3, 2021. https://asm.org/Articles/2021/August/Diagnostic-Stewardship-Interventions-That-Make-a-D
45. Clinical and Laboratory Standards Institute. Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. Approved Guideline, 4th edn. CLSI Document M39-A4. Wayne, PA, CLSI, 2014.
46. Bremmer, D. N., Trienski, T. L., Walsh, T. L., and Moffa, M. A. Role of Technology in Antimicrobial Stewardship. Med Clin North Am. 2018; 102 (5): 955-963. doi: 10.1016/j.mcna.2018.05.007
47. Thakral, Y., Sahay, S., and Mukherjee, A. Strengthening digital monitoring of antibiotic resistance in low-resource settings. J Glob Hlth. 2022; 12: 03061. doi: 10.7189/jogh.12.03061.
48. Sanchez, G. V., Fleming-Dutra, K. E., Roberts, R. M., and Hicks, L. A. Core Elements of Outpatient Antibiotic Stewardship. MMWR Recomm Rep. 2016; 65 (No. RR-6): 1–12
49. Goff, D. A., Karam, G. H., and Haines, S. T. Impact of a national antimicrobial stewardship mentoring program: Insights and lessons learned. Am J Health Syst Pharm. 2017; 74 (4): 224-231.
50. Zhu, N. J., Ahmad, R., Holmes, A., Robotham, J. V., Lebcir, R., and Atun, R. System dynamics modelling to formulate policy interventions to optimise antibiotic prescribing in hospitals. Journal of the Operational Research Society. 2020.doi:10.1080/01605682.2020.1796537
51. Sneddon, J., Afriyie, D., Sefah, I., et al. Developing a Sustainable Antimicrobial Stewardship (AMS) Programme in Ghana: Replicating the Scottish Triad Model of Information, Education and Quality Improvement. Antibiotics (Basel). 2020; 9 (10): 636. doi: 10.3390/antibiotics9100636.

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Review Article **Open Access**

Genital mycoplasmas and gynaecologic cancer: A systematic review

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Abstract:

Studies on genital mycoplasmas (GM) role in gynaecologic cancers (GC) such as cervical, endometrial, ovarian, vaginal, vulva and fallopian tube, is limited. This review was conducted to evaluate an association between GM and GC. The systematic study was conducted in accordance with PRISMA guidelines across online databases including Embase, Google Scholar, PubMed, Scopus, and Web of Science from inception to August, 2022. We included cross-sectional and case-control studies examining possible connection of GM infection and development of GC, and all evidence-based studies with likely association between GM infection and incidence of GC were studied. Selection criteria aided identification, screening, and risk of bias assessment. Thirteen studies with at least moderate risk of bias, were included. The most commonly associated GMs was *Mycoplasma genitalium* followed by *Ureaplasma urealyticum* and *Mycoplasma hominis*. Studies reported disease advancement with GMs most especially in cases of co-infection. The most associated GCs were cervical, ovarian and endometrial. Infection with *U. urealyticum, M. hominis*, and *M. genitalium* was associated with cervical cancer risk (OR 1.31-1.41), and *M. hominis* and *M. genitalium* had associated risk with ovarian (RR 0.93-1.92) and endometrial cancer (OR 1.36- 2.07). No association was found with vaginal, vulva and fallopian tube cancers.

Keywords: Genital mycoplasma; gynaecologic cancer; cervical cancer; association; infection

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Mycoplasmes génitaux et cancer gynécologique: une revue systématique

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Résumé:

Les études sur le rôle des mycoplasmes génitaux (GM) dans les cancers gynécologiques (GC) tels que ceux du col de l'utérus, de l'endomètre, de l'ovaire, du vagin, de la vulve et des trompes de Fallope sont limitées. Cette revue a été menée pour évaluer une association entre GM et GC. L'étude systématique a été menée conformément aux directives PRISMA sur des bases de données en ligne, notamment Embase, Google Scholar, PubMed, Scopus et Web of Science, du début à août 2022. Nous avons inclus des études transversales et cas-témoins examinant un lien possible avec une infection GM et le développement de GC, et toutes les études fondées sur des preuves avec une association

probable entre l'infection GM et l'incidence de GC ont été étudiées. Les critères de sélection ont facilité l'identification, le dépistage et l'évaluation du risque de biais. Treize études présentant un risque de biais au moins modéré ont été incluses. Les GM les plus couramment associés étaient *Mycoplasma genitalium* suivi de *Ureaplasma urealyticum* et *Mycoplasma hominis*. Des études ont rapporté une progression de la maladie avec les GM, plus particulièrement dans les cas de co-infection. Les GC les plus associés étaient cervical, ovarien et endométrial. L'infection par *U. urealyticum, M. hominis* et *M. genitalium* était associée au cancer du col de l'utérus (OR 1,31-1,41), et *M. hominis* et *M. genitalium* présentaient un risque associé de cancer de l'ovaire (RR 0,93-1,92) et de l'endomètre (OR 1,36-2.07). Aucune association n'a été trouvée avec le cancer du vagin, de la vulve et des trompes de Fallope.

Mots clés: Mycoplasmes génitaux; cancer gynécologique; cancer du col de l'utérus; association; infection

Introduction:

Gynaecologic cancer (GC) is reported as the most prevalent amongst other cancers in women, with significant morbidity and mortality, accounting for 25% of cancer deaths in women globally (1). Gynaecologic cancers primarily are cancers of the reproductive organs of women; cervix, vulva, vagina, uterus, ovaries and fallopian tubes. Of these, cervical, ovarian and uterine cancers are the most prevalent. Annually, 881,000 new cervical, 265,000 new ovarian and 89,300 new uterine cancer cases with deaths, are reported (2,3). The principal origins of gynaecologic cancer are still unclear, however the American Cancer Society (ACS) showed the role infectious agents in gynaecologic cancer, increasing the risk of gynaecologic cancer by 20% (3).

Cancer is usually characterized by uncontrolled growth of abnormal cells in human tissues, which are usually excessive such that they metastasize, invade and spread to other organs or tissues of the body, causing life-threatening pathological situations in most cases. Studies have attributed causes of cancer to genetics, hereditary factors and carcinogens (4,5). Microorganisms such as viruses and genital mycoplasma have been studied and their link with cervical and other forms of gynecologic cancers have been established (6-10). The potential ability of atypical bacteria such as mycoplasmas in the invasion of host cells and further promotion of cellular transmission, as a precursor to cancer pathogenesis, have since been studied from inception in the 1950s (8,11-14). *In vitro* investigations of genital mycoplasmas for potential malignancy in host cells have been carried out using animal models and human cells, resulting in variations of cell transformation (15).

Mycoplasma are bacteria species with very small size and lacking cell wall. The term 'genital mycoplasmas' (GM) denotes a group of Mycoplasma species isolated from the genitals of asymptomatic sexually active individuals of both males and females. Consequently, the relatively high prevalence of these species among asymptomatic females have been reported (16). Mycoplasmas have also been strongly associated with infertility in women of reproductive age, as they were found to be more prevalent in these group of women than in fertile women of reproductive age (17,18). Mycoplasma co-infection and its contribution to onco-pathogenesis in cervical cancer have been recently studied in Africa (18). Apart from significant association with cervical cancer (OR 1.31, 95% CI 1.61-1.49; OR 1.41, 95% CI 1.10-1.99) reported in many studies (25-28), these organisms could play a role in other gynecological cancer such as endometrial cancer with significant association ($p< 0.0001$) reported in many studies (8,10, 20-24) as well as with ovarian cancer (RR 0.93, 95% CI 0.70-1.23; RR 1.92, 95% CI 0.78-4.72) also reported in some studies (7,10,29).

Some authors have reported GM involvement in some other clinically important gynaecologic pathologies such as bacterial vaginosis (30), cervicitis (31), pelvic inflammatory disease (32,33), salpingitis, uterine myomas and endometritis (7,34). Colonization of genitals by *Mycoplasma* and *Ureaplasma* have notably been linked with human papillomavirus (HPV) and pathogenesis (7,9). Although studies have not thoroughly investigated the prevalence of such co-infections, intra-epithelial lesions have been shown to play a mediating role in this co-infection (7,29). Chief amongst the bacterial agents involved in the pathogenesis of GM-associated genital malignancy are *M. genitalium* (35), *M. hominis,* and *U. urealyticum* (7,36-38).

Current diagnosis of GCs is available for only cervical cancer which usually involves investigation for cancerous and/or pre-cancerous cervical cells. It may be advocated that Mycoplasma screening be also incorporated into test algorithms for women with suspected gynecological cancers since studies have shown strong association with presence of Mycoplasma in gynecological cancers such as ovarian cancer (39).

A good number of studies have shown a remarkable link between cancers such as gynecological cancers and genital mycoplasmas, although some of them remain inconclusive, while a few others infer that other factors may be considered in mycoplasma-mediated genital oncology investigations. Understanding the role of genital mycoplasmas in chromosomal variability in gynaecologic malignancies/cancers development

may influence development of novel approaches to their prevention. Consequently, published articles in the scientific literature regarding the involvement and specific mechanism of genital Mycoplasma infection in the development of different types of gynaecologic cancer, were systematically evaluated in this study.

Methodology:

Search approach for the study

A systematic search approach was employed in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines (40) across these scholarly databases; Embase, Google Scholar, PubMed, Scopus, and Web of Science from inception to August, 2022. The MeSH keywords used for the search included; *Mycoplasma* spp., *Ureaplasma* spp., *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma penetrans*, *Mycoplasma genitalium*, *Ureaplasma parvum*, genital mycoplasma, gynaecologic cancers, cervical, endometrial, ovarian, vulva, fallopian tube, vagina and gynaecologic tumours. The list of references for all retrieved articles similarly aided additional studies.

Systematic study selection:

The systematic selection of relevant articles included independent reviewers of two in number, to identify eligible studies using an established inclusion criteria for the study. In the instance of discrepancies, all authors considered the articles and a census reached. The inclusion criteria are; (i) journal articles published in English language and (ii) observational research articles including cross-sectional, case-control, cohort (retrospective and prospective) studies, containing study population, study type (hospital-based), type of gynaecological cancer, and genital mycoplasma infections (*Mycoplasma* spp., *Ureaplasma* spp., or both). Furthermore, review articles with systematic approach were also included.

Excluded from the study were journal articles not published in English language; congress/conference abstracts; article types such as letter to editor, case report with unstandardized methodology or inadequate data; studies with reported bacterial infections other than genital mycoplasma or viral infection in gynaecological cancer; and articles that considered an association between genital mycoplasma infection and other diseases except gynaecological cancer.

Quality assessment of included studies and data extraction:

The included studies were assessed for quality according to the Joanna Briggs Institute (JBI) critical appraisal (41). The study data assembled included characteristics such as first author, publication year, country, sample size (number of cases and/or control group), genital mycoplasma infection, frequency, diagnostic method, measures of association and participants' demographics.

Data synthesis and risk bias assessment:

Because the included studies had a low level of heterogeneity in study design, we employed a descriptive approach in analysing the data obtained. The assessment of risk of bias for included studies was done with the Cochrane Risk of Bias software with Risk of Bias of Exposures (ROBINS-E) applied for non-randomized studies (42). Each author assessed individual study within the risk of bias domain. Where discrepancy ensue, a consensus conclusion was attained. The bias risks for each domain were recorded accordingly.

Results:

Eligible studies:

Of 129 full-text articles assessed for eligibility, 116 were excluded based on the exclusion criteria. A total of 13 studies were included for data synthesis and risk bias assessment (Fig 1). Publication date for eligible studies was 1996 to 2020. Country of origin of the articles varied, with USA (n=4) having the highest number of included articles, followed by China (n=2), and Korea (n=2) (Table 1). In these studies, infections with GM were mostly assayed with polymerase chain reaction (PCR-ELISA, PCR 16SrRNA, qM-PCR and q-PCR-RT). However, culture, serology, lipid associated membrane protein enzyme immunoassay (LAMP-EIA) and Mycoplasma kit were also used. Furthermore, advanced high-throughput methodology such as metagenomic sequencing and PathoChip Array were also used.

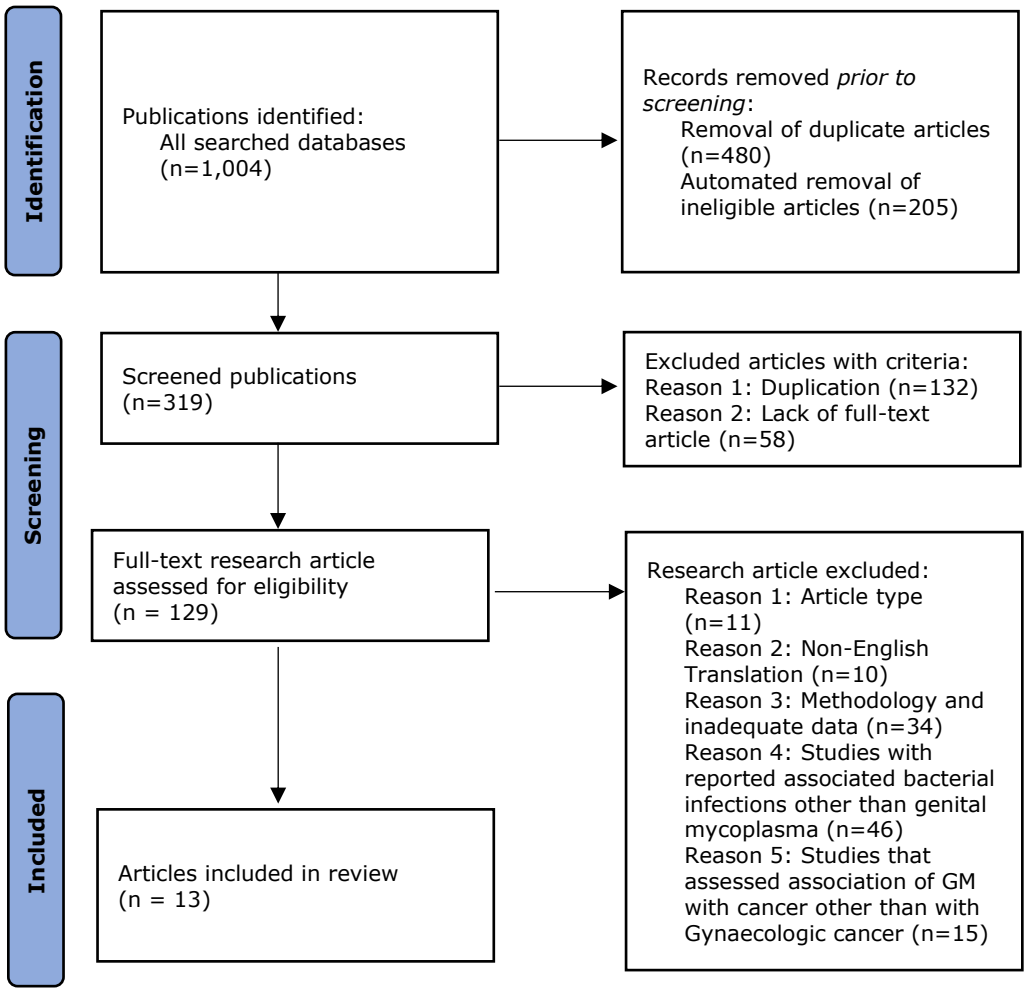

Fig 1: PRISMA flow chart with identification, screening and inclusion of articles

Associations with demographics and genital mycoplasma induced gynaecological cancer:

In the 13 included studies, data from 10,255 patients of different nationality with gynaecologic cancer (cervical, ovarian, endometrial vulva, vagina and fallopian tube) were reviewed. Patients diagnosed with GM-induced cancer were all sexually active with age ranging from 18 to 73 years. Sexually active young women (30–45 years) were also reported (19). Walther-António et al., (21) reported a significant association of GC with older women (58-71 years; $p < 0.0001$).

Other variables that could further predispose the patients to GM-induced gynaecologic cancer were also observed in the study. Walther-António et al., (21) reported significant association with post-menopausal (p=0.0034), but no association with history of diabetes (p= 0.621) and smoking status (p=0.5911). Only few authors studied these variables (Table 1).

Associations with cervical cancer:

The first cross-sectional study examined the cervical brush sample swabs of 1060 women (43). These samples were analysed using 16S metagenomic sequencing to determine the association of GM in each cervical cancer patient. Results obtained by the authors showed that cervical cancer patients with GM were significantly more prevalent in HPV patients. Abundance in *M. hominis* count significantly increased among patients with cervical cancer lesions. Overall, distribution of GM reported was *U. urealyticum* (51.4%), *M. hominis* (34%), and *M. genitalium* (2.3%) (43).

Secondly, the cervical microbiota of 4290 women (1452 HPV-positive group and 2838 HPV-negative group) were identified using molecular approach - PCR (9). The conventional

Table 1: Overview of articles included in study

Authored articles	Country	Year	Characteristics of the subjects						Sample type	Study type/ design	Sample size	Assay	GM/GC Type	Significant association
			Age range (years)	Nationality	Post-Meno-Pausal	Hypertension	Diabetes	Smoking						
Klein et al., (19)	Tanzania	2020	18-73	Tanzanian	Yes	NA	NA	NA	Cervical brush sample	HB/CS	1060	16S Metagenomics sequencing	MH, MG/CC	OR 2.1 $p < 0.0001$
Idahl et al., (44)	Germany	2020	30-81	European	Yes	NA	NA	NA	Blood samples	HB/CCS	2460	Serology	MG/OC	OR 1.36; 95% CI: 1.13–1.64
Fortner et al., (46)	USA	2019	34-81	American	Yes	NA	NA	NA	Plasma samples	HB/CCS	337	Serology	MG/OC	OR 2.07; 95% CI: 1.25–3.43; RR 1.92 (0.78-4.72)
Banerjee et al., (36)	USA	2017	NA	American	NA	NA	NA	NA	Ovarian cancer tissue	HB/CCS	99	PathoChip Array	GM/OC	$p < 0.05$
Liu et al., (9)	China	2016	18-66	Chinese	Yes	No	No	NA	Cervical brush specimen	HB/CCS	4290	PCR	UU/CC	OR 1.31 95% CI: 1.16-1.49
Walther-Antonio et al., (21)	USA	2016	≥ 18	Caucasian	Yes	Yes	No	No	Vaginal, cervical, fallopian, ovarian	HB/CS	31	16SrDNA V3-V5 region Mi-Sequecing	MH/EC	$p < 0.001$
Xiaolei et al., (26)	China	2014	20-67	Chinese	Yes	NA	NA	NA	Cervical secretion	HB/CCS	233	qPCR (Fluorescence)	UU/CC	$p = 0.002$
Debon and McGowin (25)	Korea	2014	20-70	Louisiana	Yes	NA	NA	NA	Cervical Secretion	HB/CCS	347	qPCR-RT	MG/CC	$p < 0.05$
Choi et al., (27)	Korea	2014	20-45	Korean	No	NA	NA	NA	Cervico-vaginal secretion	HB/CS	714	PCR	MH,MG, UU/CC	$p = 0.054$
Farag et al., (43)	Egypt	2013	20-48	Egyptian	No	NA	NA	Yes	Endo-cervix and posterior vaginal sample	HB/CS	300	Mycoplasma kit/Pap smear cytology	MH, UU/CC	OR 1.41-1.51; 95% CI: 1.10-1.99
Idahl et al., (45)	Sweden	2011	18-87	Swedish	Yes	NA	NA	Yes	Plasma sample	HB/CCS	118	LAMP-EIA	MG/OC	RR 0.93 95% CI: 0.70-1.23; p=0.01
Lukic et al., (28)	Italy	2006	18-50	Italian	No	NA	NA	NA	Exo-endo-cervical and vaginal sample	HB/CCS	239	Culture	UU/CC	NA
Chan et al., (37)	USA	1996	NA	NA	NA	NA	NA	NA	Achived human ovarian tisuue	HB/CCS	27	Combined PCR-ELISA	GM/OC	NA

NA = not avaliable; HB = hospital-based; CS = Cross sectional study; CCS = Case-control study; MG = *Mycoplasma genitalium*; UU = *Ureaplasma urealyticum*; MH = *Mycoplasma hominis*; GM = Genital Mycoplasma; GC = Gynecologic cancer; EC = Endometrial Cancer; OC = Ovarian Cancer; CC = Cervical Cancer; qPCR = Quantitative Polymerase chain reaction; PCR = Polymerase chain reaction; LAMP-EIA = Lipid associated membrane protein enzyme immunoassay; PCR-ELISA = Polymearse chain reaction **–** enzyme-linked immunosorbent assay

PCR result showed significant association of *U. urealyticum* in the microbiota of HPV-positive patients (AOR 1.18; 95% CI 1.04–1.34) and *U. urealyticum* were found in significantly higher frequencies (58.2%) among the HPV-positive women (9). High prevalence of *U. urealyticum* (49.3-83%) was found to be associated with the grade of cytological cervical lesions (squamous abnormalities) in 533 participants (19,26-28). *Ureaplasma urealyticum* was significantly associated (p<0.05) with risk of cervical cancer in high-grade squamous lesion (HSIL) (57.5%-65%), atypical squamous cervical cells of undetermined significance (ASCUS) (27%-30.43%) and low-grade squamous intra-epithelial lesion (LSIL) (14%-36.59%). The high presence of *U. urealyticum* in these studies is in association with HPV, which is consequently, a potential co-factor for HPV-induced precancerous and cervical cancer. *M. hominis* was found to be more prevalent than *M. genitalium* among women with cervical cancer (26,27), however, *M. genitalium* was significantly prevalent in women with severe cervical inflammation.

Association with ovarian cancer:

Mycoplasma genitalium antibodies was identified in 11.76% of 68 females with epithelial ovarian cancer using multiplex fluorescent bead-based serology (lipid-associated membrane protein-enzyme immunoassay) (44). The result showed a significant association (RR 0.93 (95% CI 0.70-1.23). A similar study found significant association (p=0.01) following detection of *M. genitalium* IgG antibodies among women with borderline ovarian tumours (45), although the association in this study was due to a type 1 error from Bonferroni correlation which reduced the significance of the finding. A significant association was reported from the analysis of plasma samples of 336 ovarian cancer patients with showed presence of MgPa-N-Terminus and MgPa antibodies of *M. genitalium* in 17% of the study population (RR 1.92, 95% CI 0.78-4.72) (46), although *C. trachomatis* seropositivity was associated with higher risk of ovarian cancer (RR 2.07, 95% CI 1.25-3.43), similar for invasive serous, and borderline tumours.

Using a molecular approach such as combined PCR-ELISA method, genital mycoplasma was detected in 59.3% of the study population using a genus-specific primer (37). Further analysis with a specie-specific primer detected *U. urealyticum* as the dominant specie. An analysis of 99 ovarian cancer tissue samples with Pan-Pathogen Array (PathoChip) technology combined with high throughput sequencing, detected an abundance (74%) of genital *Mycoplasma* spp among the microbiome of ovarian cancer patients (p <0.05) (36). However, *Mycoplasma* and *Ureaplasma* (Tenericutes) are not higher on the list of bacterial signatures in ovarian cancer when compared to Proteobacteria and Firmicutes.

Association with endometrial cancer:

The abundance of *U. urealyticum* and *M. hominis* was reported among women with benign uterine disease, uterine hyperplasia or any stage of endometrial cancer undergoing hysterectomy in a case-control study (21). Sequencing result revealed that these women had progressive PID which led to chronic inflammation and subsequently change in uterine endothelial cells (carcinogenesis).

Discussion:

This systematic review of studies intended to assess current trend of the association between GM and GC, with their aetiological and causative factors in different geographical locations. The finding associating the risk of endometrial cancer with variables such as hypertension in American women by Walther-Antonio et al., (21) may not be entirely in concurrence with other findings due to the difference in methodology adopted by the different researchers who used serology (46) and pathochip (36) as their prefered methods of analysis during their research with different study particpants from different geographical regions.

The role of *U. urealyticum* as an important factor in the promotion of gynaecological pathological processes such as cervical cancer can no longer be overlooked even at the global level, this is because studies by Choi and Roh (27) conducted in Korea, together with studies conducted with Chinese participants by Xiaolei et al., (26) were both in concurrence with a previous study conducted in Europe by Lukic et al., (28) as they all detected *U. urealytium* associated with cervical cancer. On this basis, global documentation of this organism as a gynaecological cancer-associated atypical microbe has become imperative, as it has shown to promote morbidity in women with gynaeological cytopathologies regardless of race, ethnicity and the geographical location of the patient.

Although most of the articles reviewed were mostly studies done from outside Africa, a recent study done in Africa by Klein et al., (19) with Tanzanian women as study participants using molecular methods revealed the link between *Mycoplasma* and cervical cancer in the study participants. The scarcity of African-based studies may not be unconnected with the fact that the use of molecular techniques for diagno-

stic and research purpose is a relatively new development in Black Africa and could be costly for both routine and research investigations, with scarcity of trained personnels an important factor, compared to other continent and climes (16). Another factor that may be contributory to the dearth of case-studies in sub-saharan Africa in comparison to other climes, could be the reluctance of patients in seeking hospital-based care. Many patients in Africa prefer to seek traditional remedies such as polyherbals as their primary source of care, some of these polyherbal have been proven to be contaminated above safe levels (47). Prior to this study, a previous study conducted in Egypt with women of middle-eastern origin as study subjects did not deploy molecular-based methodology, it showed a connection between cervical cancer and bacteria such as *M. hominis* and *M. genitalium*.

Scientific reviews have proven that the link between mycoplasma and cancer can no longer be considered a mere coincidence or happenstance by the global medico-scientific community, this link has been noted since 1950 when *Mycoplasma* were first noted in patients with leukemia (11). The predispostion of older and post-menopausal women as seen in this review, may also be attributed to the lowering of immunity as the women progressively age into geriatic status. It is also understood that immunocompentence dwindles as women become older (48) and may also contribute to dybiosis in the cervico-vaginal milieu of women. Dysbiosis have also been shown to be linked to many diseases such as cancer (49). Immunological factors such as pro-inflammatory cytokines which promotes inflammation in the cervicovaginal epithelium many also be upregulated during dysbiosis and displacement of friendly commensals and probiotic bacteria such as *Lactobacillus acidophilus* and *Lactobacillus crispatus* (50), who help to physiologically protect the genito-urinary tract of women from pathogenic and harmful bacteria colonization, by encouraging the growth and survival of bacteria such as mycoplasma which have been linked to some gynaecological cancers (51).

A positive concordance between molecular-based test and standard culture technique in the detected genito-urinary tract infection was noted at 98%. Notwithstanding this postulation, molecular-based test possess several features that makes it relatively beneficial to timely diagnosis and management of fastidious genito-urinary pathogens such as *Mycoplasma* and *Ureaplasm*a. This test has shown to be effective in the utilization as it can identify fastidious bacteria, provide faster assessment of antibiotic susceptibility, track drug resistance patterns and help identify factors that may affect therapy and re-infection of gynaecological microbial diseases (52,53). It is therefore hoped that since most of the articles reviewed in this study were based on molecular studies, this therefore should confer a substantial level of uniformity on the methodology of research works conducted, ensuring reproduceablity of the studies in this systematic review.

Conclusion:

Based on this review, it is understood that mycoplasma-associated gynaecoloical cancers are not specific to any geographical region and they have been detected in women of almost all race and regions, with both *Mycoplasma* and *Ureaplasma* being associated with increased risk of cervial cancer, while only *Mycoplasma* have shown association with increased risks of ovarian and endometrial cancers in women. Molecular-based test for routine investigation of genital mycoplasma in gynaecological pathologies should be encouraged in hospitals.

Contribution of authors:

CCE conceptualized the study; NRA and CVU collected and analyzed the data; IOU, CBO and ISE were involved in analysis of data; and CCE and CVN produced the manuscript draft. All authors approved the final manuscript.

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Conflict of interest:

Authors declare no conflict of interest

References:

1. Henley, S. J., Miller, J. W., Dowling, N. F., Benard, V. B., and Richardson, L. C. Uterine Cancer incidence and mortality. MMWR Morb Mortal Wkly Rep. 2018; 67: 1333-1338. doi: http://dx.doi.org/10.15585/mmwr.mm6748a1
2. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394–424. doi: 10.3322/caac.21492
3. Arbyn, M., Weiderpass, E., Bruni, L., de Sanjose, S., Saraiya, M., and Ferlay, J. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020; 8: e191-e203. doi: 10.1016/S2214-109X(19)30482-6
4. Shanmughapriya, S., Senthilkumar, G., Vinodhini, K., Das, B. C., Vasanthi, N., and Natarajaseenivasan, K. Viral and bacterial aetiologies of epithelial ovarian cancer. Eur J Clin Microbiol Infect Dis. 2012; 31: 2311–2317. doi: 10.1007/s10096-012-1570-5
5. Calle, E., and Kaaks, R. Overweight, obesity and

cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004; 4: 579–591. doi: 10.1038/nrc1408
6. Hui, Y., Tiange, S., Xi, Z., Li, L., Hou, M., and Xi, M. Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. Arch Gynecol Obstet 2018; 297: 1377-1387. doi: 10.1007/s00404-018-4733-5
7. Seyed-Abolfazl, H., Kiarash, G., Tannaz, A. J., Keikha, M., and Sahebkar, A. Association of *Chlamydia* and *Mycoplasma* infections with susceptibility to ovarian cancer: A systematic review and meta-analysis. Semin. Cancer Biol. 2022; 86: 923-928. https://doi.org/10.1016/j.semcancer.2021.07.016
8. Chase, D., Goulder, A., Zenhausern, F., Monk, B., and Herbst-Kralovetz, M. The vaginal and gastrointestinal microbiomes in gynaecologic cancers: a review of applications in etiology, symptoms and treatment. Gynecol Oncol. 2015; 138: 190–200. doi: 10.1016/j.ygyno.2015.04.036
9. Liu, J., Liu, W., Liu, Y., Zhou, X., Zhang, Z., and Sun, Z. Prevalence of microorganism coinfections in human papillomaviruses infected women in Northern China. Arch Gynecol Obstet. 2016; 293: 595–602. doi: 10.1007/s00404-015-3826-7
10. Schwabe, R., and Jobin, C. The microbiome and cancer. Nat Rev Cancer. 2013; 13: 800–812. doi: 10.1126/science.abc4552
11. Yacoub, E., Abdul-Wahab, O. M., Al-Shyarba, M. H., and Mardassi, B. B. A. The Relationship between Mycoplasmas and Cancer: Is It Fact or Fiction? Narrative Review and Update on the Situation. J Oncol. 2021; 9986550. doi: 10.1155/2021/9986550.
12. Sobstyl, M., Brecht, P., Sobstyl, A., Mertowska, P., and Grywalska, E. The Role of Microbiota in the Immunopathogenesis of Endometrial Cancer. Int J Mol Sci. 2022; 23: 5756. https://doi.org/ 10.3390/ijms23105756.
13. Klumpp, D., and Rudick, C. Summation model of pelvic pain in interstitial cystitis. Nat Rev Urol. 2008; 5: 494–500. doi: 10.1038/ncpuro1203
14. Lu, W., He, F., Lin, Z., et al. Dysbiosis of the Endometrial Microbiota and Its Association with Inflammatory Cytokines in Endometrial Cancer. Int J Cancer. 2021; 148: 1708–1716. doi: 10.1002/ijc.33428
15. Tsai, S., Wear, D. J., Shih, J. W., and Lo, S. C. Mycoplasmas and oncogenesis: persistent infection and multistage malignant transformation. Proc Natl Acad Sci. 1995; 92: 10197–10201. doi: 10.1073/pnas.92.22.10197
16. Ezeanya-Bakpa, C. C., Agbakoba, N. R., Enweani-Nwokelo, I. B., and Oguejiofor, C. B. Phylogeny-based identification of *Mycoplasma genitalium* in a Nigerian population of apparently healthy sexually active female students. Pan Afr Med J. 2022; 41: 71. doi: 10.11604/pamj.2022.41.71.19631
17. Ezeanya-Bakpa, C. C., Agbakoba, N. R., Oguejiofor, C. B., and Enweani-Nwokelo, I. B. Sequence analysis reveals asymptomatic infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* possibly leads to infertility in females: A cross-sectional study. Int J Reprod Biomed. 2021; 19: 951-958. doi: 10.18502/ijrm.v19i11.9910
18. Moridi, K., Hemmaty, M., Azimian, A., Fallah, M. H., Abyaneh, H. K., and Ghazvini, K. Epidemiology of genital infections caused by *Mycoplasma hominis, Mycoplasma genitalium* and *Ureaplasma urealyticum* in Iran; a systematic review and meta-analysis study (2000-2019). BMC Publ Health. 2020; 20: 1020. doi: 10.1186/s12889-020-08962-5
19. Klein, C., Samwel, K., Kahesa, C., et al., Mycoplasma Co-Infection Is Associated with Cervical Cancer Risk. Cancers (Basel). 2020; 12:1093. doi: 10.3390/cancers12051093
20. Yang, T. K., Chung, C. J., Chung, S. D., Muo, C., Chang, C., and Huang, C. Risk of Endometrial Cancer in Women with Pelvic Inflammatory Disease: A Nationwide Population-Based Retrospective Cohort Study. Medicine. 2015; 94: e1278. doi: 10.1097/MD.0000000000001278
21. Walther-António, M. R. S., Chen, J., Multinu, F., et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. Genome Med. 2016; 8:122. doi: 10.1186/s13073-016-0368-y
22. Mitchell, C. M., Haick, A., Nkwopara, E., et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. Am J Obstet Gynecol. 2015; 212: e1–e9. doi: 10.1016/j.ajog.2014.11.043
23. Rajagopala, S. V., Vashee, S., Oldfield, L. M., et al. The Human Microbiome and Cancer. Cancer Prev Res. 2017;10: 226–234. doi: 10.1158/1940-6207.CAPR-16-0249
24. Kyono, K., Hashimoto, T., Nagai, Y., and Sakuraba, Y. Analysis of Endometrial Microbiota by 16S Ribosomal RNA Gene Sequencing among Infertile Patients: A Single-Center Pilot Study. Reprod Med Biol. 2018; 17:297–306. doi: 10.1002/rmb2.12105
25. Dehon, P. M., and McGowin, C. L. *Mycoplasma genitalium* infection is associated with microscopic signs of cervical inflammation in liquid cytology specimens. J Clin Microbiol. 2014; 52:2398–2405. doi: 10.1128/JCM.00159-14
26. Xiaolei, C., Taot, H., Zongli, S., and Hongying, Y. The role of *Ureaplasma urealyticum* infection in cervical intraepithelial neoplasia and cervical cancer. Eur J Gynaecol Oncol. 2014; 35: 571–575.
27. Choi, Y., and Roh, J. Cervical cytopathological findings in Korean women with *Chlamydia trachomatis, Mycoplasma hominis,* and *Ureaplasma urealyticum* infections. Sci World J. 2014; 756713. doi: 10.1155/2014/756713.
28. Lukic, A., Canzio, C., Patella, A., Giovagnoli, M., Cipriani, P., and Frega, A. Determination of cervicovaginal microorganisms in women with abnormal cervical cytology: the role of *Ureaplasma urealyticum*. Anticancer Res. 2006; 26: 4843–4849.
29. Tone, A. A., Salvador, S., Finlayson, S. J., Tinker, A. N., Kwon, J. S., and Lee, C. The role of the fallopian tube in ovarian cancer. Clin Adv Hematol Oncol. 2012; 10:296–306.
30. Okoli, A. C., Agbakoba, N. R., Ezeanya, C. C., Oguejiofor, C. B., and Anukam, K, C. Comparative Abundance and Functional Biomarkers of the Vaginal and Gut Microbiome of Nigerian Women with Bacterial Vaginosis: A Study with 16S rRNA Metagenomics. J Med Lab Sci. 2019; 29: 1-26. doi: 10.5281/zenodo.4010798
31. Ezeanya, C. C., Agbakoba, N. R., Enweani, I. B., and Oguejiofor, C. B. Predominance of cervicitis agents with minimal testing rate within the student population in Benin City, Nigeria. J Obstet Gynaecol 2019; 39: 840–844. doi: 10.1080/01443615.2019.1584888
32. Silver, H. M., Sperling, R. S., St Clair, P. J., and Gibbs, R. S. Evidence relating bacterial vaginosis to intra-amniotic infection. Am J Obstet Gynecol. 1989; 161: 808–812. doi: 10.1016/0002-9378(89)90406-7
33. Hillier, S. L., Krohn, M. A., Cassen, E., Easterling, T. R., Rabe, L. K., and Eschenbach, D. A. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact

fetal membranes. Clin Infect Dis. 1995; 20: S276–S278. doi: 10.1093/clinids/20.supplement_2.s276
34. Hillier, S. L., Kiviat, N. B., Hawes, S. E., et al. Role of bacterial vaginosis-associated microorganisms in endometritis. Am J Obstet Gynecol. 1996; 175: 435–441. doi: 10.1016/s0002-9378(96)70158-8
35. Omid, Z., Simin, R., and Atefeh, M. *Mycoplasma genitalium* and Cancer: A Brief Review. Asian Pacific J Cancer Prev. 2013; 14: 3425-3428. doi: 10.7314/apjcp.2013.14.6.3425
36. Banerjee, S., Tian, T., Wei, Z., Shih, N., Feldman, M. D., and Alwine, J. C. The ovarian cancer oncobiome. Oncotarget. 2017; 8 (22): 36225–36245. doi: 10.18632/oncotarget.16717
37. Chan, P. J., Seraj, I. M., and Kalugdan, T. H., and King, A. Prevalence of mycoplasma conserved DNA in malignant ovarian cancer detected using sensitive PCR-ELISA. Gynecol Oncol. 1996; 63 (2): 258–260. doi: 10.1006/gyno.1996.0316
38. Wahid, M., Dar, S. A., Jawed, A., Mandal, R. K., Akhter, N., and Khan, S. Microbes in gynecologic cancers: Causes or consequences and therapeutic potential. Semin Cancer Biol. 2022; 86:1179-1189. doi: 10.1016/j.semcancer.2021.07.013
39. Yang, H., Zhang, J., and Shou, C. Detection of mycoplasma hyorhinis infection in ovarian cancer with in situ hybridization and immunohistochemistry. Clin Oncol Cancer Res. 2010; 7: 294–298. doi: https://doi.org/10.1007/s11805-010-0534-8
40. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I, Hoffmann, T. C., and Mulrow, C. D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021; 372: n71. doi: 10.1136/bmj.n71
41. Aromataris, E., Fernandez, R., Godfrey, C., Holly, C., Khalil, H., and Tungpunkom, P. Summarizing syste- matic reviews: methodological development, con- duct and reporting of an Umbrella review approach. Int J Evid Based Health. 2015; 13:132-140. doi: 10.1097/XEB.0000000000000055
42. Higgins, J., Morgan, R., Rooney, A., Taylor, K., Thayer, K., and Silva, R. Risk of Bias in Non-Randomized Studies of Exposure (ROBINS-E). 2022. https://www.riskofbias.info/welcome/robins-e-tool. (Accessed 19th September, 2022).
43. Farag, E., Mohamed, A., Walid, A., Azzazi, A., Fayed, S. M., and Eldin, A. K. Z. Association between genital mycoplasma and cervical squamous cell atypia. Middle East Fertil. 2013; 8:241-245. https://doi.org/10.1016/j.mefs.2013.01.003
44. Idahl, A., Le Cornet, C., Maldonado, S. G., Waterboer, T., Bender, N., and Tjonneland, A. Serologic markers of *Chlamydia trachomatis* and other sexually transmitted infections and subsequent ovarian cancer risk: results from the EPIC cohort. Int J Cancer. 2020; 147:204252. doi: 10.1002/ijc.32999
45. Idahl, A., Lundin, E., Jurstrand, M., Kumlin, U., Elgh, F., and Ohlson, N. *Chlamydia trachomatis* and *Mycoplasma genitalium* plasma antibodies in relation to epithelial ovarian tumors. Infect Dis Obstet Gynecol 2011; 2011:824627. doi: 10.1155/2011/824627
46. Fortner, R. T., Terry, K. L., Bender, N., Brenner, N., Hufnagel, K., and Butt, J. Sexually transmitted infections and risk of epithelial ovarian cancer: results from the Nurses' Health Studies. Br J Cancer. 2019; 120: 855–860. doi: 10.1038/s41416-019-0422-9
47. Udeogu, C. V., Agbakoba, N. R., and Chukwuma, G. O. Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria. Afr J Clin Exper Microbiol. 2020; 21: 354-359. doi:10.4314/AJCEM.V21I4.13
48. Haynes, L. Aging of the Immune System: Research Challenges to Enhance the Health Span of Older Adults. Front. 2020; 1: 602108. doi: 10.3389/fragi.2020.602108
49. Kinross, J. M., Darzi, A. W., and Nicholson, J. K. Gut microbiome-host interactions in health and disease. Genome Med. 2011; 3: 14. doi: 10.1186/gm228
50. Udeogu, C. V., Agbakoba, N. R., Lilian, C. N., et al. *Lactobacillus* species isolates and vaginal pro-inflammatory cytokine levels in women of reproductive age attending Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Eur J Med Sci. 2022; 4:39-44. doi:10.24018/ejmed.2022.4.6.1464
51. Kamada, N., Seo, S., Chen, G. Y., and Núñez, G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013; 13:321-335. doi: 10.1038/nri3430
52. Heytens, S., de Sutter, A., Coorevits, L., Christiaens, T., Vaneechoutte, M., and Claeys, G. Women with symptoms of a urinary tract infection but a negative urine culture: PCR-based quantification of *Escherichia coli* suggests infection in most cases. Clin Microbiol Infect. 2017; 23:647-652. doi: 10.1016/j.cmi.2017.04.004
53. Van der, Z. A., Roorda, L., Bosman, G., and Ossewaarde, J. M. Molecular Diagnosis of Urinary Tract Infections by Semi-Quantitative Detection of Uropathogens in a Routine Clinical Hospital Setting. PLoS One. 2016; 8: e0150755. doi: 10.1371/journal.pone.0150755

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Review Article **Open Access**

Microbial menace to kidney health: A review of the role of infections in acute kidney injury

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Abstract:

Acute kidney injury (AKI) of infectious aetiology is a complex condition that requires a comprehensive microbiological evaluation. This includes sepsis workup, evaluation of urinary tract infection (UTI), assessment for viral, fungal, and bacterial infections, consideration of the patient's microbiome, and vigilance towards antibiotic toxicity. Advanced molecular diagnostic tools such as metagenomic sequencing and rapid point-of-care-testing, may offer future advances in accurate and timely identification of infectious aetiologies in AKI. Careful antibiotic selection, dosing, and duration, taking into account renal function and potential toxicity, are crucial in the era of increasing antibiotic resistance. The information presented in this review were obtained through a thorough literature search using relevant search terms on various databases including PubMed, Embase, and Cochrane Library. The review identified bacterial sepsis and UTI as common infectious syndromes associated with AKI, but also emphasized the need to consider other infectious aetiologies including viral, fungal and parasitic infections in certain clinical scenarios. The review also discussed the potential role of advanced molecular diagnostic tools in identifying infectious aetiologies in AKI and the importance of careful antibiotic selection, dosing, and duration. In conclusion, a comprehensive microbiological evaluation, coupled with the use of advanced diagnostic techniques and antibiotic stewardship, is vital for the effective management of AKI from suspected infectious aetiology, which can aid optimize patient outcomes.

Keywords: Acute kidney injury; infection; sepsis; urinary tract infection; sequelae

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Menace microbienne pour la santé rénale: examen du rôle des infections dans les lésions rénales aiguës

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Résumé:

L'insuffisance rénale aiguë (IRA) d'étiologie infectieuse est une affection complexe qui nécessite une évaluation microbiologique complète. Cela comprend le bilan de sepsis, l'évaluation de l'infection des voies urinaires (IVU), l'évaluation des infections virales, fongiques et bactériennes, la prise en compte du microbiome du patient et la vigilance à l'égard de la toxicité des antibiotiques. Les outils de diagnostic moléculaire avancés, tels que le séquençage métagénomique et les tests rapides au point d'intervention, pourraient offrir de futures avancées dans l'identification précise et rapide des étiologies infectieuses de l'IRA. Une sélection, un dosage et une durée minutieux des antibiotiques, en tenant compte de la fonction rénale et de la toxicité potentielle, sont cruciaux à l'ère de la résistance croissante aux antibiotiques. Les informations présentées dans cette revue ont été obtenues grâce à une recherche documentaire approfondie à l'aide de termes de recherche pertinents dans diverses bases de données, notamment PubMed, Embase et Cochrane Library. L'examen a identifié la septicémie bactérienne et l'infection urinaire comme des syndromes infectieux courants associés à l'IRA, mais a également souligné la nécessité de prendre en compte d'autres étiologies infectieuses, notamment les infections virales, fongiques et parasitaires, dans certains scénarios cliniques. L'examen a également discuté du rôle potentiel des outils de diagnostic moléculaire avancés dans l'identification des étiologies infectieuses de l'AKI et de l'importance d'une sélection, d'un dosage et d'une durée minutieux des antibiotiques. En conclusion, une évaluation microbiologique

complète, associée à l'utilisation de techniques de diagnostic avancées et à la gestion des antibiotiques, est essentielle pour la prise en charge efficace de l'AKI due à une étiologie infectieuse suspectée, ce qui peut contribuer à optimiser les résultats pour les patients.

Mots clés: Lésion rénale aiguë; infection; état septique; infection urinaire; séquelles

Introduction:

Acute kidney injury (AKI) is a complex clinical syndrome that is characterized by a sudden elevation in serum creatinine levels, a reduction in urine output, or a combination with this rapid decline in kidney function leading to the accumulation of metabolic waste products and a range of clinical manifestations and outcomes (1). AKI is however always diagnosed in the context of the patient as it is contextually a clinical diagnosis (2). The World Health Organization (WHO) has identified AKI as a global health priority due to its significant associated morbidity, mortality, and healthcare costs, especially as it is now the 10th leading cause of death globally (3).

In recent years, the role of infectious agents in causing AKI has been increasingly recognized, leading to a growing interest in the microbiological aetiology of this condition especially for sepsis associated AKI (SA-AKI) which has extremely high mortality (4-7). The UTI-associated AKI (UTI-AKI) is another increasingly described syndrome with a multifactorial pathogenesis (8). There are numerous methods to categorize AKI. However, the conventional approach involves classifying AKI based on urine output and changes in creatinine levels, which can be further categorized as prerenal, intrinsic renal, or postrenal (1). Syndromic classification is increasingly becoming more popular as physicians find it more clinically relevant. The syndromic classification captures the underlying pathophysiology and include the nephrotoxic, hepatorenal, and cardiorenal AKIs (1,9-11).

To successfully manage AKI, it is essential to accurately diagnose the underlying cause, including potential microbiological factors. This usually requires a thorough clinical assessment, relevant laboratory tests (such as blood and urine cultures), and imaging to identify potential sources of infection or inflammation (12). Precise diagnosis can guide targeted therapeutic strategies, such as administering appropriate antibiotics for bacterial infections or treating underlying factors for inflammation or injury. Therefore, it's important to have a good understanding of the possible microbiological and infectious causes of AKI for effective clinical management.

Given the significant impact of infectious agents on kidney health and patient outcomes, understanding the microbiological aetiology of AKI is crucial. Here, we provide an overview of the microbiological aetiology of AKI, with a focus on the role of infectious agents. This study reviewed the epidemiology of AKI caused by infectious agents, highlighting the different pathogens responsible for this condition and their modes of transmission. Additionally, the study delves into the pathogenesis of AKI caused by various infectious agents, including the mechanisms by which they can cause kidney damage and the factors that contribute to disease severity. The study concludes by discussing the various diagnostic techniques used to determine the cause of AKI and also covers the treatment and management approaches for AKI resulting from infectious cases.

Methododology:

A comprehensive literature search was conducted using various databases, including PubMed, Embase, and Cochrane Library, to identify relevant articles related to the microbiological evaluation of AKI with suspected infectious aetiology. The search was conducted using MESH terms such as "acute kidney injury," "AKI", "sepsis-associated AKI", "urinary tract infections associated AKI", "viral infections associated AKI", "bacterial infections associated AKI", "fungal infections associated AKI", and "microbiome in AKI". The search was limited to English language articles published from January 1980 to April 2023.

Relevant articles were identified through a systematic process that included screening of article titles, abstracts, and full texts. Additional articles were identified through manual searching of reference lists of relevant articles. Inclusion criteria for the review were articles that discussed the microbiological aspects of AKI with suspected infectious aetiology including sepsis-related AKI, UTI-related AKI, viral, fungal, and bacterial infections. Articles that discussed the diagnosis, treatment, and antibiotic stewardship in AKI with suspected infectious aetiology were also included. Data extraction and synthesis were performed independently by two reviewers. Any discrepancy was resolved through discussion and consensus.

Results and Discussion:

Infectious syndromes as aetiology of AKI

1. Urinary tract infections and AKI

Urinary tract infection (UTI) refers to primarily bacterial infection occurring any-

where from the urethral meatus to the perinephric fascia and kidneys (13). They are a relatively significant cause of AKI and can lead to significant morbidity and mortality if left untreated. In general, UTIs are more common in women than in men, with up to 60% of women experiencing a UTI at some point in their lifetime (14,15). To initiate UTI, the bacteria typically ascend the urethra into the bladder and can cause cystitis, pyelonephritis, or both. In severe cases, the bacteria can enter the bloodstream and cause sepsis, which is a significant risk factor for AKI.

The UTI-associated AKI (UTI-AKI) is increasingly being recognized as an important syndrome with associated high morbidity and mortality. The pathogenesis of UTI-AKI is multifactorial and involves both direct and indirect mechanisms. The direct mechanisms include bacterial invasion of the renal parenchyma, leading to pyelonephritis, interstitial nephritis, and/or glomerulonephritis. The indirect mechanisms include sepsis-induced hypotension, intrarenal vasoconstriction, and/or tubular obstruction by cellular debris or crystals (8). Immune-mediated injury, or the activation of pro-inflammatory cytokines also contributes to kidney injury (8,16).

The most common pathogens associated with UTI are Gram-negative bacteria (17). Of these, *Escherichia coli* is the most commonly implicated and accounts for up to 80% of community-acquired infections (18-21). Other Gram-negative bacteria such as *Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa*, are also common causes of UTI. Gram positive organisms such as *Enterococcus faecalis* and *Staphylococcus saprophyticus* can also cause UTI, especially in older adults and sexually active women. These bacteria express virulence factors, which promote inflammation and tissue damage. These include lipopolysaccharide, adhesins, and toxins. Acute kidney injury can occur as a complication of UTI in some cases. The mechanism of AKI due to UTI is complex and can involve several factors enumerated below.

Infection-related inflammation:

Urinary tract infection, particularly pyelonephritis which is an upper UTI, can cause inflammation of the kidneys. Inflammatory processes triggered by the infection can lead to damage to the kidney tissue, including the renal tubules. Inflammation can also result in increased permeability of blood vessels in the kidney, leading to leakage of fluid and proteins into the kidney tissue and impairing normal kidney function (22). Predisposing factors to development of UTI-AKI include advancing age, diabetes mellitus, upper UTI, afebrile status, and impaired baseline renal function (23). Advanced age is a known risk factor for UTI-associated AKI, as elderly patients may have reduced physiological reserves and increased vulnerability to infections. Diabetes mellitus predisposes patients to UTI-related AKI due to compromised immune function and impaired renal perfusion (23).

Furthermore, upper UTIs, which involve the kidneys pose a higher risk of AKI compared to lower UTIs (22). Additionally, afebrile UTI patients may not exhibit typical signs of infection, leading to delayed diagnosis and treatment, which can increase the risk of AKI. Lastly, patients with impaired baseline renal function such as those with pre-existing chronic kidney disease, may be more susceptible to UTI-related AKI due to reduced renal reserve (13). Therefore, vigilance and close monitoring is required in management of UTI especially in immunocompromised patients to avoid progression, thus preventing further renal damage and improving patient outcomes.

Obstruction:

UTI can cause obstruction in the urinary tract, which can lead to backflow of urine and increased pressure in the kidneys. This can disrupt normal kidney function and impair blood flow to the kidneys, leading to AKI. Obstruction can be caused by factors such as urinary stones, blood clots, or swelling of the urinary tract due to infection (24).

Toxins produced by bacteria*:*

Some bacteria that cause UTIs can produce toxins which can directly damage the kidney tissue. For example, certain strains of *E. coli*, which are a common cause of UTIs, can produce Shiga toxin that can cause kidney injury (25,26).

Immune response:

AKI may also be brought on by the immunological response to UTIs (27). The immune system may generate inflammatory mediators in reaction to the infection, which can harm kidney tissue and decrease kidney function (27-29).

Host factors:

Individual patient factors, such as age, overall health status, and pre-existing kidney disease, can also influence the development of AKI in the setting of UTI. Patients with pre-existing kidney disease or other risk factors for AKI may be more susceptible to kidney injury due to UTI (1).

It is important to note that not all UTIs will result in AKI, and the risk of AKI due to UTI varies depending on multiple factors. Prompt diagnosis and appropriate treatment of UTIs are important to prevent complications such as AKI. Treatment of UTI-related AKI involves the use of antibiotics to eradicate the bacterial infection and supportive measures such as hydration and electrolyte manage-

ment (1). In severe cases, renal replacement therapy may be necessary (1). Prevention of UTIs can be achieved through good hygiene practices, adequate fluid intake, and prophylactic antibiotic therapy in high-risk individuals.

2. Sepsis-associated AKI

The Surviving Sepsis Campaign 2016 International guidelines define sepsis as a life-threatening organ dysfunction caused by dysregulated host response to infection, spreads throughout the body (30). Sepsis is a leading cause of AKI in critically ill patients (31). The most commonly implicated bacterial species implicated in sepsis include *E. coli, K. pneumoniae,* and *P. aeruginosa* (32-34). Viral, fungal, and parasitic causes of sepsis are relatively uncommon (34). Respiratory, gastrointestinal, genitourinary, and skin or soft tissue infections are the most common foci of sepsis, accounting for more than 80% of cases (30,34). Urinary tract infections more commonly lead to culture positive sepsis. The pathogenesis of UTI-AKI is thought to differ from non-UTI-AKI but is also complex and multifactorial (35).

Sepsis results in widespread release of cytokines and other inflammatory mediators, which ultimately results in extensive inflammation and damage to various organs, including the kidneys where tubular cell injury and necrosis results in decline in glomerular filtration rate and ultimately lead to AKI (31,35-37). Previous assumption has been that hypotension causing hypoperfusion of kidneys was the major cause of AKI in sepsis, however, recently it has been proven that microvascular dysfunction with release of inflammatory mediators, cytokines, microparticles with adaptation of tubular cells, is the major contributor of sepsis induced AKI (31). It has also been suggested that patients with UTI-AKI may respond differently to interventions and have outcomes different from patients with non-UTI-AKI (1).

The severity of AKI in sepsis can vary, but it is often associated with a high mortality rate. In addition, sepsis-related AKI has been linked to an increased risk of developing chronic kidney disease (CKD) and end-stage renal disease (ESRD) (1,35). Therefore, early recognition and treatment of sepsis are critical in preventing the development of AKI and its associated complications. The management of sepsis-related AKI involves addressing the underlying infection, optimizing hemodynamic status, and providing supportive care, such as renal replacement therapy when necessary (30,31). Antibiotic therapy is the cornerstone of sepsis management and should be initiated as soon as possible after diagnosis (30,34).

3. Viral infections and AKI

Viral infections have been identified as potential causes of acute kidney injury AKI. The viruses that have been associated with AKI include adenovirus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis B and C viruses, and human immunodeficiency virus (HIV) (38-42). Direct viral invasion of kidney cells is one mechanism by which certain viruses can cause AKI. For example, viruses like cytomegalovirus (CMV) and adenovirus can directly invade renal tissue, leading to inflammation, necrosis, and dysfunction of the kidneys (41,43). This can result in direct damage to the renal cells and structures, leading to AKI. Immune-mediated injury is another mechanism by which viral infections can lead to AKI. Viral infections can trigger an immune response that results in inflammation and immune-mediated injury to the kidneys (44,45). This can occur through the formation of immune complexes during the viral infection that deposit in the kidneys, leading to glomerulonephritis.

Haemodynamic instability is another significant factor in the development of AKI during viral infections. Severe viral infections can cause hemodynamic instability such as hypotension, sepsis, or shock, which can reduce blood flow to the kidneys and result in ischemic injury (46). Reduced blood flow can impair the normal functioning of the kidneys and contribute to the development of AKI. Systemic inflammation is also a key mechanism by which viral infections can cause AKI. Viral infections can trigger a systemic inflammatory response that can directly or indirectly damage renal tissue (47,48). This can occur through the release of inflammatory mediators and cytokines during the viral infection, leading to inflammation and injury to the kidneys (38).

Rhabdomyolysis is another mechanism by which viral infections can contribute to AKI. Certain viral infections can cause muscle breakdown, resulting in the release of myoglobin into the bloodstream. Myoglobin can then accumulate in the kidneys, leading to myoglobin-induced kidney injury and potentially contributing to the development of AKI (49). Thrombotic microangiopathy is also a mechanism by which certain viral infections can cause AKI (50). Viral infections, such as human immunodeficiency virus (HIV), can cause widespread clotting of small blood vessels, impairing renal blood flow and leading to AKI (50,51).

Identifying the viral aetiology of AKI requires a thorough evaluation, including a detailed patient history, physical examination, and laboratory investigations. Serological testing for viral antibodies, viral nucleic acid test-

ing (such as polymerase chain reaction), and viral antigen detection are commonly used methods for identifying the viral cause of AKI. In some cases, renal biopsy may be necessary to confirm the viral aetiology of AKI (52,53). Viral infections can thus cause AKI through various mechanisms, including direct cytopathic effects, immune complex deposition, and drug toxicity. Although rare, AKI should be considered in patients with viral infections, especially those who are immunocompromised or have other risk factors for AKI.

4. Fungal infections and AKI

Fungal infections are increasingly recognized as a potential cause of acute kidney injury (AKI), particularly in immunocompromised individuals (54). Fungal infections can cause AKI through various mechanisms, including direct invasion of renal tissue, immune-mediated injury, thrombotic microangiopathy, and obstructive manifestations. Direct invasion of renal tissue by fungi can result in tissue damage and inflammation, leading to AKI (54). Fungal species such as *Candida*, *Aspergillus* and *Cryptococcus* can invade the renal tissue, leading to inflammation, necrosis, and dysfunction of the kidneys (55,56). This can result in direct damage to renal cells and structures, leading to AKI.

Immune-mediated injury is another mechanism by which fungal infections can lead to AKI (57). Fungal infections can trigger an immune response that results in inflammation and immune-mediated injury to the kidneys. This can occur through the formation of immune complexes or hypersensitivity reactions during the fungal infection, leading to glomerulonephritis or interstitial nephritis, which can contribute to the development of AKI (54,57).

Thrombotic microangiopathy is another mechanism by which fungal infections can cause AKI. Fungal infections, such as invasive aspergillosis, can cause widespread clotting of small blood vessels, impairing renal blood flow and leading to AKI (58-60). The formation of microclots in the renal vasculature can cause thrombotic microangiopathy, a condition characterized by microvascular thrombosis, platelet aggregation, and endothelial injury (61, 62). Obstructive manifestations of fungal infections can also lead to AKI (61,63). Fungal infections can cause obstruction in the urinary tract, leading to obstructive uropathy and subsequent renal dysfunction. Fungal elements can form mycotic balls or fungal balls in the renal pelvis, ureters, or bladder, leading to obstruction and impaired urine flow, which can result in AKI.

Diagnosing fungal infections as the cause of AKI requires a high index of suspicion and careful evaluation. A thorough patient history, physical examination, and laboratory investigations, including blood cultures, fungal serology, and imaging studies, may be necessary to identify the fungal aetiology of AKI. In some cases, renal biopsy may be required to confirm the presence of fungal elements in renal tissue.

Management of fungal-associated AKI involves prompt removal of any indwelling urinary catheters or other foreign bodies, as well as the initiation of appropriate antifungal therapy (12,64). In cases of obstructive uropathy, urgent decompression of the collecting system with placement of a nephrostomy tube may be necessary to preserve renal function. In severe cases, renal replacement therapy may be required until the patient's underlying infection is controlled and renal function recovers. Understanding the mechanisms by which fungal infections can cause AKI, including direct invasion, immune-mediated injury, thrombotic microangiopathy, and obstructive manifestations, is crucial for accurate diagnosis and management of AKI associated with fungal infections.

Role of microbiome in AKI

The human microbiome, which consists of trillions of microorganisms residing in and on the body, has been recognized as a crucial factor in human health and disease (65,66). Emerging evidence suggests that alterations in the microbiome can impact the development and progression of AKI. The microbiome can play a role in AKI through several mechanisms. First, dysbiosis, or an imbalance in the composition and function of the microbiome, can lead to the production of harmful metabolites or toxins that can directly damage renal tissue and impair renal function (67). For example, gut dysbiosis can result in increased production of trimethylamine-N-oxide (TMAO), a metabolite associated with cardiovascular and renal dysfunction, which can contribute to the development of AKI (68,69).

The microbiome can also influence the host immune response, leading to immune-mediated injury in the kidneys. Dysbiosis can trigger an abnormal immune response, leading to systemic inflammation and immune-mediated injury to renal tissue. This can result in inflammation, tissue damage, and impaired renal function, contributing to the development and progression of AKI (70,71). The microbiome can affect the systemic and renal hemodynamics (65,72). Microbial metabolites, such as short-chain fatty acids, can modulate renal blood flow, vascular tone, and blood pressure, which can have direct effects on renal function (73). Alterations in the microbiome can disrupt these regulatory mechanisms, leading to haemodynamic instability and contributing to AKI. Dysbiosis can affect the microbiome's capacity to control the imm-

une response and modify the host's reaction to sepsis (74). In septic individuals, this may lead to organ failure, systemic inflammation, and AKI .

An active area of research is figuring out how the microbiota affects AKI, and interventions targeting the microbiome may hold promise as potential therapeutic strategies for AKI. Strategies such as probiotics, prebiotics, faecal microbiota transplantation, and dietary interventions are being explored to modulate the microbiome and potentially mitigate the risk or severity of AKI.

Approach to management of patients with microbial-associated AKI

In the context of AKI with suspected infectious aetiology, a systematic and comprehensive microbiological workup is imperative for appropriate diagnosis and management. This includes a step-wise approach that encompasses sepsis workup, evaluation of UTI (complicated and uncomplicated), assessment for viral, fungal, and bacterial infections, consideration of the patient's microbiome and vigilance towards antibiotic toxicity.

Sepsis workup:

Suspected sepsis requires expedited blood cultures to identify the causative microorganism(s) and guide timely initiation of appropriate antibiotic therapy (31). Multiple blood cultures from different sites should be obtained prior to initiating antibiotics, if feasible, to maximize the likelihood of positive culture results. Concurrent laboratory tests such as complete blood count, coagulation studies, and inflammatory markers aid in determining the severity and progression of sepsis.

UTI work up:

A urine culture with sensitivity testing should be performed in all suspected cases of UTIs with AKI. Quantitative urine cultures are preferred for accurate results (75,76). Urine culture identifies the specific microorganisms responsible and facilitates tailored antibiotic selection. In complicated UTIs, additional imaging studies such as computerized tomographic (CT) or ultrasound scan may be necessary to evaluate for urinary tract abnormalities or abscesses.

Viral, fungal, and bacterial workup:

In certain clinical scenarios, viral, fungal, and bacterial infections may need to be considered. For instance, in immunocompromised patients, viral infections such as CMV or polyomavirus may be suspected. Risk factors such as recent antibiotic use or indwelling urinary catheters may prompt consideration of fungal infections caused by *Candida*. Extended-spectrum beta-lactamase (ESBL) testing may be warranted in cases of suspected antibiotic-resistant bacterial infections.

Other factors worth considering:

Antimicrobial therapy, in addition to other supportive therapies, may be required to effectively manage AKI. Renal replacement therapy (RRT) is a common intervention used in cases of severe AKI or when conservative management fails to improve renal function. RRT includes haemodialysis, peritoneal dialysis, and continuous renal replacement therapy. Other supportive therapies may include electrolyte replacement, blood pressure management, and nutritional support.

Prevention:

In addition to contributing to emergence of antibiotic-resistant bacteria that can cause serious infections that can result in AKI, antibiotic abuse and misuse can also cause renal impairment. However, when used appropriately, antibiotics can prevent development of AKI (77,78). AKI-causing diseases such as hepatitis and COVID-19 can be prevented by vaccinations. For proper kidney function to be maintained, adequate water intake is essential as AKI can develop as a result of dehydration, especially in persons who are already at higher risk due to underlying medical disorders.

Early recognition and management of infections that can lead to AKI are critical in preventing the progression of the disease. Timely diagnosis and appropriate treatment of infections can help reduce the risk of complications and improve outcomes in people with AKI. It is important to note that the comprehensive management of AKI requires a multidisciplinary approach, involving physicians, nurses, pharmacists, and other healthcare professionals. The management of AKI should be guided by the underlying cause, severity of the condition, and the patient's clinical status.

Conclusion and future direction:

There is still much to learn about the pathophysiology of AKI caused by infectious pathogens, despite progress in diagnosis and treatment. To find novel biomarkers that can help in the early identification and diagnosis of AKI brought on by infectious pathogens, more study is required. Additionally, more research is required to examine the possible application of novel therapeutics, such as immunomodulatory drugs and stem cell therapies, for the prevention and treatment of AKI.

Furthermore, for the development of targeted therapeutics that can successfully treat and prevent this disorder, it will be essential to comprehend the interaction between infectious pathogens and the immune system in the pathogenesis of AKI. To address this significant public health concern and enhance

outcomes for patients with AKI brought on by infectious agents, doctors, researchers, and public health officials must work together. The emerging understanding of how the microbiome influences the risk and severity of AKI is another significant advance. Modulation of the microbiome may be a viable strategy for preventing and treating AKI.

Despite recent advances, much remains to be done to improve the prevention, diagnosis, and management of AKI caused by infectious agents. Future research should focus on developing new strategies for preventing infections, identifying novel biomarkers for early detection and monitoring of AKI, and exploring the role of the microbiome in AKI pathogenesis. By addressing these challenges, we can hope to reduce the burden of AKI and improve outcomes for affected patients.

Contributions of authors:

AB conceived the review idea, designed the outline, wrote the aspects of abstract and conclusion, and reviewed the manuscript; MN searched the literature for relevant publications, wrote the aspects of introduction, methodology, infectious syndromes, patient approach, and future directions. Both authors approved the final manuscript.

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References:

1. Bellomo, R., Kellum, J., and Ronco, C. Acute kidney injury. 2012; 380: 756–766.
2. Kellum, J. A., and Lameire, N. The definition of acute kidney injury. 2018; 391 (10117): 202–203.doi:10.1016/S0140-6736(17)31630-6
3. Noncommunicable diseases. (n.d.). https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases
4. Chua, H. R., Wong, W. K., Ong, V. H., et al. Extended Mortality and Chronic Kidney Disease After Septic Acute Kidney Injury. J Intensive Care Med. 2020; 35 (6): 527–535. doi: 10.1177/0885066618764617
5. Peerapornratana, S., Manrique-Caballero, C. L., Gómez, H., et al. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int. 2019; 96 (5): 1083. doi: 10.1016/J.KINT.2019.05.026
6. Poston, J. T., and Koyner, J. L. Sepsis associated acute kidney injury. BMJ. 2019; 364. doi: 10.1136/BMJ.K4891
7. Gomez, H., and Kellum, J. A. Sepsis-induced acute kidney injury. Curr Opin Crit Care. 2016; 22 (6): 546. doi: 10.1097/MCC.0000000000000356
8. Królicki, T., Bardowska, K., Kudla, T., et al Acute kidney injury secondary to urinary tract infection in kidney transplant recipients. Sci Rep. 2022; 12 (1): 10858. doi:10.1038/S41598-022-15035-7
9. Simonetto, D. A., Gines, P., and Kamath, P. S. Hepatorenal syndrome: pathophysiology, diagnosis, and management. 2020; 370: m2687. doi: 10.1136/BMJ.M2687
10. Lullo, L. Di, Reeves, P. B., Bellasi, A., et al, Cardiorenal Syndrome in Acute Kidney Injury. Semin Nephrol. 2019; 39 (1): 31–40. doi:10.1016/J.SEMNEPHROL.2018.10.003
11. Uber, A. M., and Sutherland, S. M. Nephrotoxins and nephrotoxic acute kidney injury. Pediatr Nephrol. 2020; 35 (10): 1825–1833. doi: 10.1007/S00467-019-04397-2
12. Mercado, M. G., Smith, D. K., and Guard, E. L. Acute Kidney Injury: Diagnosis and Management. Am Fam Physician. 2019; 100 (11): 687–694. https://www.aafp.org/pubs/afp/issues/2019/1201/p687.html
13. Miller, O., and Hemphill, R. R. Urinary Tract Infection and Pyelonephritis. Emerg Med Clin North Am. 2001; 19 (3): 655–674. doi: 10.1016/S0733-8627(05)70208-X
14. Foxman, B. Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. Am J Med. 2002; 113 (1 SUPPL. 1): 5–13. doi: 10.1016/S0002-9343(02)01054-9
15. Iregbu, K., Medugu, N., Abdullahi, N., et al. Urine culture contamination: a one-year retrospective study at the National Hospital, Abuja. Afr J Clin Exper Microbiol. 2013; 14 (2): 101–104. doi: 10.4314/ajcem.v14i2.10
16. Turgut, F., Awad, A. S., and Abdel-Rahman, E. M. Acute Kidney Injury: Medical Causes and Pathogenesis. J Clin Med. 2023; 12 (1): 12. doi: 10.3390/JCM12010375
17. Chu, C. M., and Lowder, J. L. Diagnosis and treatment of urinary tract infections across age groups. Am J Obstet Gynecol. 2018; 219 (1): 40–51. doi: 10.1016/j.ajog.2017.12.231
18. Medugu, N., Aworh, M. K., Iregbu, K., et al. Molecular characterization of multi drug resistant *Escherichia coli* isolates at a tertiary hospital in Abuja, Nigeria. Sci Rep. 2022; 12 (1). doi: 10.1038/s41598-022-19289-z
19. Kunin, C. M. Urinary tract infections in females. Clin Infect Dis. 1994; 18 (1): 1–12. doi:10.1093/CLINIDS/18.1.1
20. Kubik, M. J., and McCarter, Y. S. Controversies in the diagnosis of urinary tract infections. Clin Microbiol Newsletter. 2012; 34 (23): 185–191. doi:10.1016/J.CLINMICNEWS.2012.11.001
21. Medugu, N., Tickler, I. A., Duru, C., et al. Phenotypic and molecular characterization of beta-lactam resistant Multidrug-resistant Enterobacterales isolated from patients attending six hospitals in Northern Nigeria. Sci Rep. 2023; 13 (1). doi: 10.1038/S41598-023-37621-Z
22. Woodrow, G., Patel, S., Berman, P., et al. Asymptomatic acute pyelonephritis as a cause of acute renal failure in the elderly. Postgrad Med J. 1993; 69(809): 211. doi: 10.1136/PGMJ.69.809.211
23. Hsiao, C. Y., Yang, H. Y., Hsiao, M. C., et al. Risk factors for development of acute kidney injury in patients with urinary tract infection. PLoS One. 2015;10(7).doi:10.1371/JOURNAL.PONE.0133835
24. Heyns, C. F. Urinary tract infection associated with conditions causing urinary tract obstruction and stasis, excluding urolithiasis and neuropathic bladder. World J Urol. 2012; 30 (1): 77–83. doi: 10.1007/S00345-011-0725-9
25. Obrig, T. G., and Karpman, D. Shiga toxin pathogenesis: Kidney complications and renal failure. Curr Top Microbiol Immunol. 2012; 357: 105–136.doi:10.1007/82_2011_172/COVER
26. Karpman, D., Sartz, L., and Johnson, S. Pathophysiology of typical hemolytic uremic syndrome. Semin Thromb Hemost. 2010; 36 (6): 575–585. doi:10.1055/S-0030-1262879
27. Abraham, S. N., and Miao, Y. The nature of immune responses to urinary tract infections. Nat Rev Immunol. 2015; 15 (10): 655. doi: 10.1038/NRI3887

28. Brennan, E., Kantharidis, P., Cooper, M. E., et al. Pro-resolving lipid mediators: regulators of inflammation, metabolism and kidney function. Nat Rev Neph. 2021; 17 (11): 725–739. doi: 10.1038/s41581-021-00454-y
29. Donaldson, T. A. Immune responses to infection. Crit Care Nurs Clin North Am. 2007; 19(1): 1–8. doi: 10.1016/J.CCELL.2006.10.001
30. Avni, T., Lador, A., Lev, S., et al. Sepsis: Diagnosis and Management. Am Fam Physician. 2020; 101 (7): 409–418. doi: 10.1371/JOURNAL.PONE.0129305
31. Prowle, J. R. Sepsis-associated AKI. Clin J Am Soc Nephrol. 2018; 13 (2): 339–342. doi:10.2215/CJN.07310717
32. Medugu, N., and Iregbu, K. C. Trends in profiles of bacteria causing neonatal sepsis in Central Nigeria Hospital. Afr J Clin Exper Microbiol. 2016; 18 (1): 49. doi: 10.4314/ajcem.v18i1.7
33. Medugu, N., Iregbu, K., Tam, P.-Y. I., et al. Aetiology of neonatal sepsis in Nigeria, and relevance of group B streptococcus: A systematic review. PLoS One. 2018; 13 (7). doi: 10.1371/journal.pone.0200350
34. Bullock, B., and Benham, M. D. Bacterial Sepsis. 2022. https://www.ncbi.nlm.nih.gov/books/NBK537054/
35. Bellomo, R., Kellum, J., Ronco, C., et al. Acute kidney injury in sepsis. Intensive Care Med. 2017; 43: 816–828.
36. Egi, M., Ogura, H., Yatabe, T., et al. The Japanese Clinical Practice Guidelines for Management of Sepsis and Septic Shock 2020 (J-SSCG 2020). J Intensive Care. 2021; 9 (1). doi: 10.1186/s40560-021-00555-7
37. Bagshaw, S. M., and Bellomo, R. Urine abnormalities in acute kidney injury and sepsis. Contrib Nephrol. 2010; 165: 274–283.
38. Legrand, M., Bell, S., Forni, L., et al. Pathophysiology of COVID-19-associated acute kidney injury. Nat Rev Nephrol. 2021; 17 (11): 751-764. doi:10.1038/s41581-021-00452-0
39. Watanabe, T. Hemolytic uremic syndrome associated with influenza A virus infection. Nephron. 2001; 89 (3): 359–360. doi: 10.1159/000046102
40. Watanabe, T. Renal complications of seasonal and pandemic influenza A virus infections. Eur J Pediatr. 2013; 172 (1): 15–22. doi:10.1007/S00431-012-1854-X/TABLES/1
41. Sujeet, K., Vasudev, B., Desai, P., et al. Acute kidney injury requiring dialysis secondary to adenovirus nephritis in renal transplant recipient. Transplant Infect Dis. 2011; 13 (2): 174–177. doi:10.1111/J.1399-3062.2010.00577.X
42. Wong, F. Treatment to Improve Acute Kidney Injury in Cirrhosis. Curr Treat Options Gastroenterol. 2015; 13 (2): 235–248. doi: 10.1007/S11938-015-0050-2
43. Lachiewicz, A. M., Cianciolo, R., Miller, M. B., et al. Adenovirus causing fever, upper respiratory infection, and allograft nephritis complicated by persistent asymptomatic viremia. Trans Infect Dis. 2014; 16 (4): 648–652. doi:10.1111/TID.12248
44. Jang, H. R., and Rabb, H. Immune cells in experimental acute kidney injury. Nat Rev Nephrol. 2015; 11(2): 88–101. doi: 10.1038/nrneph.2014.180
45. Zheng, L., Gao, W., Hu, C., et al. Immune cells in ischemic acute kidney injury. Curr Protein Pept Sci. 2019; 20: 770–776.
46. Fani, F., Regolisti, G., Delsante, M., et al. Recent advances in the pathogenetic mechanisms of sepsis-associated acute kidney injury. J Nephrol. 2018; 31 (3): 351–359. doi:10.1007/S40620-017-0452-4/METRICS
47. Becker, R. C. COVID-19-associated vasculitis and vasculopathy. J of Thrombosis and Thrombolysis. 2020;50 (3): 499– 511. doi: 10.1007/s11239-020-02230-4
48. Lin, L., Wang, X., Ren, J., et al Risk factors and prognosis for COVID-19-induced acute kidney injury: a meta-analysis. BMJ Open. 2020; 10 (11).doi:10.1136/bmjopen-2020-042573
49. Watanabe, T. Rhabdomyolysis and acute renal failure in children. Pediatr Nephrol. 2001; 16(12): 1072–1075.doi:10.1007/s004670100030
50. George, J. N., and Nester, C. M. Syndromes of Thrombotic Microangiopathy. N Engl J Med. 2014; 371 (7): 654–666. doi:10.1056/NEJMRA1312353
51. Emlet, D. R., Shaw, A. D., and Kellum, J. A. Sepsis-Associated AKI: Epithelial Cell Dysfunction. Semin Nephrol. 2015;35 (1): 85–95. doi: 10.1016/j.semnephrol.2015.01.009
52. Kudose, S., Batal, I., Santoriello, D., et al. Kidney biopsy findings in patients with COVID-19. J Am Soc Nephrol. 2020; 31(9):1959–1968. doi: 10.1681/asn.2020060802
53. Ferlicot, S., Jamme, M., Gaillard, F., et al. The spectrum of kidney biopsies in hospitalized patients with COVID-19, acute kidney injury, and/or proteinuria. Nephrol Dial Transplant. 2021; 36 (7): 1253–1262. doi: 10.1093/ndt/gfab042
54. Gandhi, B. V., Bahadur, M. M., Dodeja, H., et al. Systemic fungal infections in renal diseases. J Postgrad Med, 51 (5): 30. https://www.jpgmonline.com/article.asp?issn=0022-3859;year=2005;volume=51;issue=5;spage=30;epa ge=36;aulast=Gandhi
55. Sobel, J. D., Fisher, J. F., Kauffman, C. A., et al. Candida urinary tract infections - Epidemiology. Clin Infect Dis. 2011; 52 (SUPPL. 6). doi: 10.1093/CID/CIR109
56. Parasuraman, R., and Julian, K. Urinary tract infections in solid organ transplantation. Am J Transplant. 2013; 13 (SUPPL.4): 327 – 336. doi: 10.1111/ajt.12124
57. Raghavan, R., Date, A., and Bhaktaviziam, A. Fungal and Nocardial infections of the kidney. Histopathology. 1987; 11 (1): 9–20. doi:10.1111/J.1365-2559.1987.TB02605.X
58. Cicek, N., Yildiz, N., Kadayifci, E. K., et al. Invasive aspergillosis in a patient with end stage renal disease. Med Mycol Case Rep. 2017; 18: 12–14.doi:10.1016/j.mmcr.2017.06.006
59. Patterson, T. F., Thompson, G. R., Denning, D. W., et al. Practice guidelines for the diagnosis and management of aspergillosis: Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016; 63(4): e1-60. doi: 10.1093/cid/ciw326
60. Schwartz, S., and Thiel, E. Clinical presentation of invasive aspergillosis. Mycoses. 1997; 40 (Suppl 2): 21–24. doi:10.1111/j.1439-0507.1997.tb00559.x
61. Ullah, S. R., Jamshaid, A., and Zaidi, S. Z. Renal aspergilloma presenting with pelvi-ureteric junction Obstruction (PUJO). J Pak Med Assoc. 2016; 66(7): 903–904.
62. Paul, S., Singh, V., Sankhwar, S., and Garg, M. Renal aspergillosis secondary to renal instrumentation in immunocompetent patient. BMJ Case Rep. 2013; 11: 2013:bcr2013200306. doi: 10.1136/bcr-2013-200306
63. Sahlén, A. O., Suvarna, S. K., and Wilkie, M. E. A case of invasive pulmonary aspergillosis in renal failure. Nephrol Dial Trans. 2004; 19 (10): 2687. doi: 10.1093/ndt/gfh418
64. Jo, S. K., Rosner, M. H., and Okusa, M. D. Pharmacologic treatment of acute kidney injury: why drugs haven't worked and what is on the horizon. Clin J Am Soc Nephrol. 2007; 2: 356–365.
65. Lim, E. S., Wang, D., and Holtz, L. R. The Bacterial Microbiome and Virome Milestones of Infant Development. Trends Microbiol. 2016; 24 (10): 801–810. doi: 10.1016/j.tim.2016.06.001
66. Usman, S. F., Shuaibu, I. R., Durojaiye, K., et al. The presence of microorganisms in follicular fluid and its effect on the outcome of *in vitro* fertilization-embryo transfer (IVF-ET) treatment

cycles. PLoS One. 2021; 16: e0246644. doi: 10.1371/journal.pone.0246644
67. Lei, J., Xie, Y., Sheng, J., and Song, J. Intestinal microbiota dysbiosis in acute kidney injury: novel insights into mechanisms and promising therapeutic strategies. Ren Failure. 2022; 44 (1): 571. doi:10.1080/0886022X.2022.2056054
68. Xu, K. Y., Xia G. H., Lu, J. Q., et al. Impaired renal function and dysbiosis of gut microbiota contribute to increased trimethylamine-N-oxide in in chronic kidney disease patients. Sci Rep. 2017; 7(1):1445.doi:10.1038/s41598-017-01387-y
69. Zhang, W., Miikeda, A., Zuckerman, J., et al. Inhibition of microbiota-dependent TMAO production attenuates chronic kidney disease in mice. Sci Rep. 2021; 11 (1): 518 doi: 10.1038/S41598-020-80063-0
70. Singbartl, K., Bishop, J., Wen, X., et al. Differential effect of kidney-lung cross-talk during acute kidney injury and bacterial pneumonia. Kidney Int. 2011; 80: 633-644.
71. Dellepiane, S., Marengo, M., and Cantaluppi, V. Detrimental cross-talk between sepsis and acute kidney injury: new pathogenic mechanisms, early biomarkers and targeted therapies. Crit Care. 2016; 20 (1): 61. doi: 10.1186/s13054-016-1219-3
72. Yatsunenko, T., Rey, F. E., Manary, M. J., et al. Human gut microbiome viewed across age and geography. Nature. 2012 486 (7402): 222–227. doi: 10.1038/NATURE11053
73. Foresto-Neto, O., Ghirotto, B., and Câmara, N. O. S. Renal Sensing of Bacterial Metabolites in the Gut-kidney Axis. Kidney. 2021; 360 (29): 1501–1509. doi: 10.34067/KID.0000292021
74. Putignani, L. Human gut microbiota: onset and shaping through life stages and perturbations. Front Cell Infect Microbiol. 2012; 2: 144. doi:10.3389/FCIMB.2012.00144/BIBTEX
75. Bacheller, C. D., and Bernstein, J. M. Urinary tract infections. Med Clin of N Amer. 1997; 81(3): 719–730.doi:10.1016/S0025-7125(05)70542-3
76. Patton, J. P., Nash, D. B., and Abrutyn, E. Urinary tract infection: Economic considerations. Med Clin of N Amer. 1991; 75 (2): 495–513. doi: 10.1016/S0025-7125(16)30466-7
77. Joyce, E. L., Kane-Gill, S. L., Fuhrman, D. Y., et al. Drug-associated acute kidney injury: who's at risk? Pediatr Nephrol. 2017; 32 (1): 59–69. doi: 10.1007/s00467-016-3446-x
78. Nwajiobi-Princewill, P., Medugu, N., Gobel, M., et al. Using longitudinal antibiotic point prevalence survey (PPS) to drive antimicrobial stewardship programmes in a Nigerian tertiary hospital. Afr J Clin Exper Microbiol. 2021; 22(2): 284–289. doi: 10.4314/ajcem.v22i2.22

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Review Article **Open Access**

A review of the legal and ethical perspectives in HIV/AIDS management in Nigeria

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Abstract:

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) remain major public health issues in Nigeria and other developing countries. Discrimination even among healthcare workers (HCWs), which includes poor service delivery at the point-of-care and human rights abuses, are the main factors that continue to hinder HIV eradication in developing countries, and these spread across all levels of HIV/AIDS services, from counseling and testing, to treatment and care. People living with HIV/AIDS (PLWHA) have continued to suffer from unethical conduct, human rights abuses, discrimination, and stigmatization from HCWs, employers of labor, educational institutions, religious houses, and the public. There exist Federal and some State laws that protect the rights and privileges of PLWHA, prevent discrimination and stigmatization from the general public, prevent employers from discriminating against persons with HIV infection, protect workers who criticize hazardous conditions in the workplace, and offer compensation to victims of HIV-related human rights abuses and employees for contracting job-related diseases. However, HIV-related human rights abuses, stigmatization, and discrimination, have continued unabated, not because there are no laws to protect victims, but due to ignorance of the law, complicated by the fact that some existing laws have remained dormant with regard to implementation and enforcement. Domestication of these laws by various State Governments in the country and enforcement by relevant institutions are also big issues. It is imperative for healthcare professionals to be aware of current professional standards and the general public to be aware of laws protecting victims of the virus.

Keywords: HIV/AIDS; human rights abuses; discrimination; stigmatization; law

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Un examen des perspectives juridiques et éthiques dans la gestion du VIH/SIDA au Nigeria

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Résumé:

L'infection par le virus de l'immunodéficience humaine (VIH) et le syndrome d'immunodéficience acquise (SIDA) restent des problèmes de santé publique majeurs au Nigeria et dans d'autres pays en développement. La discrimination, même parmi les travailleurs de la santé (TS), qui comprend une mauvaise prestation de services au point d'intervention et des violations des droits de l'homme, sont les principaux facteurs qui continuent d'entraver l'éradication du VIH dans les pays en développement, et ces facteurs se propagent à tous les niveaux du VIH/SIDA services, du conseil et du test au traitement et aux soins. Les personnes vivant avec le VIH/SIDA

(PVVIH) ont continué de souffrir de comportements contraires à l'éthique, de violations des droits humains, de discrimination et de stigmatisation de la part des travailleurs de la santé, des employeurs, des établissements d'enseignement, des maisons religieuses et du public. Il existe des lois fédérales et certaines lois étatiques qui protègent les droits et privilèges des PVVIH, préviennent la discrimination et la stigmatisation de la part du grand public, empêchent les employeurs de discriminer les personnes infectées par le VIH, protègent les travailleurs qui critiquent les conditions dangereuses sur le lieu de travail et offrent une indemnisation aux victimes des violations des droits de l'homme liées au VIH et des employés qui ont contracté des maladies liées au travail. Cependant, les violations des droits humains, la stigmatisation et la discrimination liées au VIH se poursuivent sans relâche, non pas parce qu'il n'existe pas de lois pour protéger les victimes, mais en raison de l'ignorance de la loi, compliquée par le fait que certaines lois existantes sont restées en sommeil avec en ce qui concerne la mise en œuvre et l'application. La domestication de ces lois par les différents gouvernements des États du pays et leur application par les institutions compétentes constituent également des problèmes majeurs. Il est impératif que les professionnels de santé connaissent les normes professionnelles en vigueur et que le grand public soit informé des lois protégeant les victimes du virus.

Mots-clés: VIH/SIDA; Abus des droits de l'homme; discrimination; stigmatisation; loi

Introduction:

Some years ago, people could not come out publicly to talk about their HIV status. Today, while there have been tremendous achievements on the journey as far as HIV/AIDS awareness is concerned, we must recognize the fact that much more still needs to be done to ensure that people living with and affected by HIV and AIDS (PLWHA) have the right to access care and to live free from HIV-related stigma and discrimination (1). Human immuno deficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) remain major public health issues in Nigeria (1).

Acts of stigmatization and discrimination from healthcare workers (HCWs), which include poor service delivery at the point-of-care and human rights abuses, as well as from the general public, are the main factors that continue to hamper HIV eradication in Nigeria. These acts spread across all levels of HIV/AIDS services, from counseling and testing, to treatment and care, thereby discouraging people from getting tested and treated (2).

The 2015 UNAIDS guideline on terminologies defines stigma as a set of beliefs or attitudes designating a person or group of individuals as undeserving or dishonorable (3). Discrimination is a consequence of stigma when any form of division, exclusion, or restriction is exhibited against any individual because of certain personal attributes or characteristics. For effective and operational purposes, HIV-related stigma is labeled as negative beliefs, feelings and attitudes toward PLWHA (4).

Although stigma has been significantly reduced by training and retraining of service providers, poor or lack of access to HIV/AIDS care services, especially lack of access to antiretroviral drugs and other supportive care, complicated by poor attitude of HCWs towards PLWHA, has remained a huge burden on fighting HIV and AIDS to finish, resulting in high cases of HIV/AIDS-related deaths in some developing countries (2). Disciplinary laws against homosexuals and injection drug users (IDUs) have caused difficulties in accessing HIV care services for many reasons which include fear of disclosing their lifestyle and status to healthcare professionals (5).

The commonest approach targeted at reducing HIV stigma and discrimination in Nigeria is media campaigns and public awareness aimed at educating the public about HIV and the eventual reduction of stigma and discrimination. Other strategies include measures put in place for counseling, testing, and treatment (2). The main philosophy of these approaches was that if HIV is seen as a chronic condition like diabetes and hypertension, the infection would be well humanized thereby reducing stigma and discrimination against people infected and affected by HIV. Two other programs related to HIV stigma in Nigeria were the PLWHA Stigma Index (SI) measurement (6), and the Legal Environment Assessment (LEA) in the HIV response (7).

This review is aimed at examining relevant laws and legislations as well as existing medical professional ethics that protect the rights and privileges of PLWHA, and also create general awareness among healthcare professionals, PLWHA and the public.

Brief overview of HIV/AIDS:

Background:

Human immunodeficiency virus (HIV), the causative agent of AIDS, is a single-stranded RNA retrovirus that attacks specific white blood cells with CD4 receptor on their surface (8). The virus is classified into types, groups, subtypes, and sub-subtypes according to its genetic variety (8). HIV type 1 (HIV-1) is extensively distributed worldwide and can be further divided into four genetic groups; group M *(major)*, group O (*outlier*), group N (*new* or *non-M, non-O*), and the newly categorized group P (8). Whereas HIV-1 groups N, P, and O are limited to countries of the Central Africa, mainly Cameroon, HIV-1 group M is responsible for the AIDS pandemic, being responsible for more than 90% of worldwide HIV infections (9). HIV-2 is restricted to the West African countries (10). Nine subtypes of HIV-1 group M are presently known (A–D, F–H, J, and K). Some subtypes are further characterized into

sub-subtypes, for example, subtype A into A1, A2, and A3, and subtype F into F1 and F2 (10).

HIV can be transmitted through sex, needles, unsterilized instruments, unscreened blood transfusions, and other routes. The virus destroys the $CD4^+$ cells, weakening infected person's immune system against opportunistic infections such as tuberculosis and fungal infections, severe bacterial infections, and some cancers (11). Globally it is estimated that 36.7 million people are living with HIV/AIDS and 25.5 million of these are in sub-Saharan Africa (12). Every individual who may be at risk for HIV should have access to testing, according to the WHO. It is important that HIV testing services follow the 5Cs; consent, confidentiality, counseling, correct results, and connection with treatment and other supportive services (11).

Clinical presentation of HIV infection:

In the first few months following infection, many persons do not exhibit any HIV-related symptoms and may not even be aware that they are infected. Flu-like symptoms such as fever, sneezing, headache, and sore throat could be early symptoms in others. However, the virus is most contagious during these initial few months (11). As the disease progresses, symptoms will be more pronounced, and these may include swollen lymph nodes, weight loss, fever, diarrhea, and cough. HIV impairs the body's capacity to fight other infections, and without treatment, persons with HIV will become more vulnerable to other severe illnesses such as tuberculosis, bacterial infections, cryptococcal meningitis, and some malignancies such as lymphomas and Kaposi's sarcoma (11).

Rapid test kits can be used to diagnose HIV and they offer immediate results, but a laboratory test is still necessary to confirm the diagnosis. Early detection increases treatment options and lowers the chance of spreading to others, such as sexual partners and needle-sharing partners (11).

Prevention and treatment:

HIV transmission is fully preventable. Effective treatment with antiretroviral therapy (ART) prevents transmission from mother to child during pregnancy, delivery, and breastfeeding. An infected person on antiretroviral therapy and virally suppressed (viral load less than 1000 copies per milliliter) will not transmit HIV to their sexual partners. (11). The use of condoms prevents HIV and other sexually transmitted infections, and prophylactic use of antiretroviral medicine prevents HIV. Circumcision of males is advised in high-burden African nations. Harm reduction (needle syringe programs and opioid substitution therapy) prevents HIV and other blood-borne infections in people who inject drugs (12).

HIV is treated with antiretroviral drugs which are usually a combination of more than one drug. While ART cannot cure HIV, it slows down blood-borne viral reproduction and reduce the viral load to undetectable levels. ART enables PLWHA to live healthy and productive lives. It also serves as an efficient preventative measure, lowering the probability of transmission by 96% (12). As soon as feasible after an HIV diagnosis, people should be offered and connected to antiretroviral treatment (ART). Counseling on adherence to ART and to stop engaging in practices that encourage transmission, and periodic monitoring using clinical and laboratory parameters are paramount (12).

Plights of HIV victims and resultant ethico-legal issues:

In Nigeria and other developing countries, the Fundamental Human Rights (FHRs) of PLWHA are constantly violated with reckless abandon in several ways, such as testing without consent, refusal of treatment, and lack of confidentiality (5). Testing without consent appears to be common practice in many private and public healthcare settings, for example, pregnant women who attend antenatal care (ANC) are often tested for HIV without counseling and without their consent (13). Testing without consent also occurs in pre-employment screening and pre-admission medical fitness tests in educational institutions, and at times testing for research purposes, are violations of the FHRs of these individuals (14).

Medical ethics is a system of moral principles that spread across values and clinical judgments with regard to the practice of medicine and medical research allowing for people, regardless of ethnicity, religion or gender, to receive high-quality ethical care. Four commonly accepted principles of healthcare ethics include respect for autonomy, principle of non-maleficence, beneficence, and justice (14). Autonomy refers to the patient's right to decide what happens to his/her own body, whereas confidentiality refers to the patient's health information being kept confidential and not disclosed to a third party without the patient's consent (15). Beneficence requires the medical practitioner to act in the best interest of the patient. Many ethical and legal issues may arise in an effort to prevent or control the further spread of the virus (15).

The duty of confidentiality of HCWs to their patients is not usually upheld in cases involving PLWHA. Where people fear forced testing or disclosure of their health status without their consent, they will avoid HIV counseling, testing, and support because these are likely to mean that they will face stigma, discrimination, and other negative effects of the breach

of such duty (15). The FHRs of PLWHA are violated when they are subjected to degrading or dehumanizing treatment simply because of their HIV status. (14) The instances where an employer denies a person employment because of his HIV status, a health worker refuses to treat a person living with the virus, friends and family avoid him/her like a plague, or a person's movement is restricted all because of his HIV status, all amount to stigmatization and discrimination, and a violation of their rights and privileges guaranteed under the law (15).

Legal and Ethical Perspectives:

1. Fundamental Human Rights by the Nigerian Constitution:

Fundamental Human Rights (FHRs) are a set of worldwide entitlements that persons enjoy irrespective of their gender, culture, religion, culture, nationality or other status that are protected and proclaimed by both local and international laws. In Nigeria, the provisions on FHRs are enshrined in chapter 4 (sections 33 to 44) of the 1999 Constitution of the Federal Republic of Nigeria (16). They are not to be violated, but where they are violated, the plaintiff (victim) can seek redress in a court of law. Hence, as for every other person, the FHRs of PLWHA are well protected by the Constitution (16).

Section 33 (RIGHT TO LIFE) states; "Every person has a right to life, and no one shall be deprived intentionally of his life"; Section 34 (RIGHT TO DIGNITY OF HUMAN PERSON) states "Every individual is entitled to respect for the dignity of his person, and accordingly, no person shall be subjected to torture or to inhuman or degrading treatment"; Section 35 (RIGHT TO PERSONAL LIBERTY) states "Every person shall be entitled to his personal liberty and no person shall be deprived of such liberty"; Section 37 (RIGHT TO PRIVACY) states "The privacy of citizens, their homes, correspondence, telephone conversations, and telegraphic communications is hereby guaranteed and protected"; and Section 42 (RIGHT TO FREEDOM FROM DISCRIMINATION) states "No citizen of Nigeria shall be subjected to any form of discrimination and deprivation".

One of the major challenges of FHRs as enshrined in the constitution is the fact that FHRs are not absolute. According to Section 45 sub-section 1 of the Constitution, "Nothing in Sections 37, 38, 39, 40, and 41 shall invalidate any law that is substantially justifiable in a democratic society; (a) in the interest of defense, public safety, public order, public morality, or public health, or (b) for the purposes of protecting the rights and freedom of other persons". This provision provides grounds upon which certain FHRs can be violated and one of such grounds is public health. It therefore goes without saying that where the disclosure of the HIV status of a person would be in the better interest of the public, such disclosure will not be in contravention of the constitutionally guaranteed right to privacy of the individual.

2. The Child Rights Act (CRA)

The Child's Rights Act (2003) is the law that guarantees the rights of all children in Nigeria including children affected by HIV/AIDS (17). Children as defined by the Child's Rights Act (2003) are any person under the age of 18, and this group is usually victims of abuse and exploitation in all forms. The National Human Rights Commission (NHRC) has a mandate to promote, protect, and enforce the rights of all citizens as well as foreign nationals in Nigeria and undertake several procedures of promoting and protecting the rights of children under this age group because they are vulnerable.

Section 1 states that "In every action concerning a child, whether undertaken by an individual, private or public body, service or institutions, court of law, or administrative or legislative authority, the best interest of the child shall be the primary consideration" (17). Section 2 (A child to be given protection and care necessary for his/her well-being) states "A child shall be given such protection and care as is necessary for the well-being of the child, taking into account the rights and duties of the child's parents, legal guardians, or other individuals, institutions, services, agencies, organizations or bodies legally responsible for the child" (17). "Every person, institution, service, agency, organization, and body responsible for the care or protection of children shall conform with the standards established by the appropriate authorities, particularly in the areas of safety, health, welfare, number and suitability of their staff and competent supervision" (17).

Section 3 (Application of Chapter IV of the 1999 Constitution) states "The provisions in Chapter IV of the Constitution of the Federal Republic of Nigeria 1999, or any successive constitutional provisions relating to Fundamental Rights, shall apply as if those provisions are expressly stated in this Act" (17). However, this very important law is yet to enjoy universal acceptance across Nigeria, thereby making implementation and enforcement difficult. So far, only 24 out of 36 States of the Federation have adopted the CRA as a State law.

3. The HIV/AIDS Anti-discrimination Act

The HIV/AIDS (Anti-discrimination) Act was passed into law in the year 2014. It is divided into four parts and has 31 sections (18). The purpose of the Act is to protect the

rights and dignity of PLWHA. Section 1 states "(a) eliminating all forms of discrimination based on HIV status; (b) fostering an environment of acceptance so that people with HIV/AIDS can continue to work in regular settings for as long as they are deemed medically fit to do so; (c) promoting appropriate and effective ways of managing HIV in the workplace, community, institutions, and other fields of human endeavor; (d) establishing conditions that are supportive and safe for everyone to work in and learn; (e) achieving balance between each person's obligations and rights in society; and (f) putting into practice the commitments outlined in Chapter 4 of the Federal Republic of Nigeria's 1999 Constitution, as amended, and other international and regional human rights" (18).

Section 2 of the Act states that; (a) This Act applies to all persons living with and affected by HIV and AIDS in Nigeria; (b) The Nigerian Armed Forces, Nigerian Police, State Security Services, other paramilitary organizations, schools, hospitals, and places of worship, and other employers of labor and employees in both the public and private sectors of the country, are covered by this Act (18).

Section 8 of the Act, deals with the issue of disclosure; "(a) Prior to accessing any public or privately delivered services, employment and any other opportunity, no individual or institutions shall necessitate an individual to reveal his or her HIV status or the status of other persons, by asking questions, directly or indirectly; (b) In spite of the restrictions in this section, any spouse or roommate who believes they may be in danger of contracting HIV from a partner has the right to know their partner's HIV status" (18)..

Section 9(1) states that "No employer, institution, or individual will require a test of HIV as a prerequisite for employment, access to private or public services or opportunities, except where it is shown, on the certification of two competent medical authorities (working independently), that failure to take such a test constitutes a clear and present danger of HIV transmission to others". Section 9 (2) states "No educational institution may demand HIV testing as part of its standard medical screening procedures for student admission or accreditation" (18).

Section 21 (1) of the Act, 2014, orders that "an employer employing five or more persons shall in consultation with the employees or their representatives adopt a written workplace policy that is consistent with the National HIV/AIDS workplace policy for the working environment" (18). This Act was passed into law in Nigeria in 2014, but has since continued to be dormant owing to non-implementation. It was enacted to discourage discrimination against PLWAHA. It is therefore meaningless that a law that was enacted to stop the discrimination of victims of HIV has been dormant as if it does not even exist.

The HIV/AIDS anti-discrimination Act 2014 specifically gave the duty to ensure compliance with the entire HIV/AIDS (Anti-Discrimination) Act, 2014, to the office of the Attorney-General of the Federation. Now the problem is, whether the office of the Attorney-General of the Federation is even aware of this Act and why it has not performed its duty of ensuring the implementation of the law and compliance by institutions of learning and employers of labor in Nigeria. It is also worrisome that an Act that was enacted about nine years ago is yet to be domesticated by many States of the Federation, hence making implementation and enforcement difficult.

4. The Patients' Bill of Rights (PBoR)

People living with HIV, like every other person, are protected under the Patients' Bill of Rights (PBoR), especially with regard to access to emergency healthcare. The PBoR was launched by the Federal Government of Nigeria on August 1, 2018 (19) and is a law developed by the Consumer Protection Council (CPC) in conjunction with the Federal Ministry of Health to protect consumers of health services in Nigeria. It sums up the existing rights of patients in the Constitution of the Federal Republic of Nigeria, the Consumer Protection Act, the Child Rights Act, the Freedom of Information Act, the National Health Act, other regulations, and professional ethical codes such as the Hippocratic Oath.

The PBoR lists the patients' rights and responsibilities, and health providers' responsibilities toward these rights. It makes the constitutional right to life more sensitive to everyone (19). There are 12 Rights that every patient is entitled to, according to the document; (a) Right to relevant information, (b) Right to timely access to medical records, (c) Right to transparent billing, (d) Right to privacy, (e) Right to clean healthcare environment, (f) Right to be treated with respect, (g) Right to receive urgent care, (h) Right to reasonable visitation, (i) Right to decline care, (j) Right to decline or accept to participate in medical research, (k) Right to quality care, and (l) Right to complain and express dissatisfaction regarding services received.

As with other very important Federal laws, domestication by many States of the Federation is a big issue, even though the Federal Competition and Consumer Protection Commission (FCCPC) Act empowers the body to activate the provisions of Section 17(3) (d) of the Constitution**,** for State-owned or private healthcare facilities and providers, which, enjoins the State to ensure that "there are satisfactory medical and health services for all individuals". Sadly, apart from the general mandate of ServiCom and the FCCPC on con-

sumer issues, no government agency is specifically responsible for the tracking and implementation of the PBoR.

5. The Code of Medical Ethics

According to Section 1 and subsection 2(c) of the Medical and Dental Practitioners Act (CAP 221), Law of the Federal Republic of Nigeria, 1990 (Decree No. 23, 1988), one of the statutory duties of the Medical and Dental Council of Nigeria (MDCN) is to periodically review and prepare a statement about the code of conduct that the Council considers desirable for the practice of the profession in Nigeria, known as the Code of Medical Ethics (20).

Rule 24 of the Code of Medical Ethics (22) gives a guideline on the MANAGEMENT OF HIV/AIDS AND OTHER SOCIALLY DREADED INFECTIOUS DISEASES and therefore states that "The prevalence of highly hazardous (contagious) ailments should be noted by practitioners. It is therefore worthy of note that practitioners should in no way discriminate in handling and treating such patients, that they maintain appropriate confidentiality, and apply a multi-disciplinary approach. Such patients should only be referred on the precise basis of professional competence" (21).

"The psychological and social consequences associated with HIV/AIDS, hepatitis B, Lassa fever, Ebola fever and others should be up in the minds of practitioners handling such cases" (21). "Practitioners should ensure that they are not used as agents by employers or others to deny infected patients their jobs where there is no clinical indication for removal of such employees from their jobs" (21). It becomes ethical for the practitioner to provide pre-and post-test counseling when investigations are clinically justified (21). Consent is not required when a patient is the one looking for a diagnosis. However, if an investigation is needed only for screening or for research, informed consent is necessary (21).

The Legal Environment Assessment (LEA)

The goal of the HIV Legal Environment Assessment (LEA) is to advance a greater understanding of the nation's laws and policies, particularly as they relate to how they affect the rights of HIV-positive people, the vulnerability of individuals to HIV, as well as the implications of related laws for individuals and organizations that work on HIV/AIDS (22). The LEA aims to determine which laws and practices have the ability to lessen or increase HIV-related stigma, which laws safeguard against discrimination, and which laws can provide access to justice through legal redress of HIV-related discrimination experiences (22). It is believed that a thorough assessment of the legal and policy environment will help strengthen the response system to HIV/AIDS in the country (22).

The LEA is part of the drive at international and regional levels to use laws as tools for HIV elimination. To improve equity in access to services among key populations, the law and regulatory environment must not be prohibitive (2). The UN General Assembly favors the conduct of LEA. The importance of the law in responses to HIV was stressed at its Special Session on HIV/AIDS in 2001 as well as the Political Declarations of 2006 and 2011, including law reform, community education, and enforcement mechanisms (22). Recommendations made to countries include commitment to intensifying national efforts by creating enabling legal, social, and policy frameworks in each national context to eliminate stigma, discrimination, and violence related to HIV; promote access to HIV prevention, treatment, care, and support and non-discriminatory access to education, health care, employment, and social services, as well as provide legal protections for people affected by HIV, including inheritance rights and respect for privacy and confidentiality; and promote and protect all human rights and fundamental freedoms with particular attention to all people vulnerable to and affected by HIV.

The LEA reveals the following; (a) The experiences of abuse of human rights of people living with and affected by HIV/AIDS as well as key and affected populations; (b) The high cost of and lengthy litigation process hinder progress in seeking redress; (c) The existence of a weak legal environment for an effective human rights-based response to HIV/AIDS; and (d) The need for sensitization, and community mobilization for the popularization of provisions of the Anti-Discrimination Act and the corresponding laws (22).

The LEA in Nigeria was therefore designed to identify and review existing laws, regulations, and policies that could impact the national HIV response through varieties of qualitative research methodologies, such as desk review, focus group discussion, in-depth interviews, and key informant interviews (7). Findings showed that the legal environment in Nigeria is weak for effective human rights-based response to HIV/AIDS (7). While advocating for the review and domestication of many of the existing laws related to HIV/AIDS victims and their care, the LEA report also called for legal literacy among key populations and stakeholders in the health and justice sectors (2).

Conclusion & Recommendation:

People living with and affected by HIV/AIDS have continued to suffer from unethical conducts, human rights abuses, discrimination

and stigmatization, from healthcare workers, employers of labor, educational institutions, religious houses, and the general public, majorly due to ignorance of the law. Although Federal and some State laws already exist that protect the rights and privileges of PLHIV, prevent discrimination and stigmatization from the general public, prevent employers from discriminating against PLWHA, protect workers who criticize hazardous conditions in the workplace, and offer compensation to victims of HIV-related human rights abuses and employees for contracting a job-related disease, HIV-related human rights abuses, stigmatization, and discrimination, have continued unabated. This is not because there are no laws to protect victims, but due to ignorance of the law, complicated by the fact that some of the laws have remained dormant with regard to implementation and enforcement. Domestication of these laws by various state governments is also a big issue. Because several of these issues have remained unsettled, it is imperative for healthcare workers to be aware of current professional standards and the public to be aware of laws protecting victims of the virus.

Based on the principle of *ignorantia iuris non est excusatio* (ignorance of the law is not an excuse), it is extremely important for healthcare professionals to be guided always by the ethics of the profession and also be aware of all relevant laws protecting the rights of people living with and affected by HIV and AIDS. Although Medical Ethics is part of medical education, medicolegal principles should be incorporated into medical education for medical students as a course on its own and part of continuing medical education for medical practitioners. Hospitals in Nigeria should set up medicolegal departments to be headed by a medical practitioner who has a special interest in medical law.

The Federal Government through the office of the Attorney General of the Federation should take steps to ensure full implementation and enforcement of all the laws protecting victims of HIV-related human rights abuses. State Governments in Nigeria that are yet to domesticate all the relevant laws should do so as a matter of urgency towards relieving the plight of victims of HIV-related human rights abuses. People living with and affected by HIV/AIDS as well as the general public, should be aware of their rights and privileges. This can be achieved by translating all the relevant laws into local languages and circulating them through the mass media. There is a need to educate and enlighten law enforcement agents to be aware of these laws and enforce them accordingly.

Contributions of authors:

OHK, SBA, AOB, ABT, ONE, OOS, and OSO conceived the idea; OHK developed and reviewed the legal aspect of the work. All the authors discussed and approved to the final manuscript.

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References:

1. National Agency for the Control of AIDS. Global AIDS Response Country Progress Report. NACA, Abuja, Nigeria, 2015.
2. Odimegwu, C. O., Akinyemi, J. O., and Alabi, O. O. HIV-stigma in Nigeria: review of research studies, policies, and programmes. AIDS Research and Treatment, 2017
3. UNAIDS. UNAIDS Terminologies Guidelines, Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland, 2015.
4. UNAIDS, Reduction of HIV-related stigma and discrimination: Guidance note, Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland, 2014
5. Onotai, L. O., Nwaorgu, O. G., and Okoye, B. C. Ethical issues in HIV/AIDS infections. Nig J Med. 12005; 3 (3): 282-285
6. HIV Leadership through Accountability Programme: GNP+; NEPWHAN. PLHIV Stigma Index Nigeria Country Assessment. Amsterdam: GNP+, 2011.
7. National Agency for the Control of AIDS. Legal Environment Assessment for HIV/AIDS Response in Nigeria, NACA, Abuja, Nigeria, 2015.
8. Robertson, D. L., Anderson, J. P., Bradac J. A., et al. HIV-1 nomenclature proposal. Science. 2000; 288 (5463): 55–57
9. Tebit D. M., and Arts, E. J. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. Lancet Infect Dis. 2011; 11 (1): 45–56
10. Eholié, S., and Anglaret, X. Commentary: decline of HIV-2 prevalence in West Africa: good news or bad news? Int J Epidemiol. 2006; 35 (5): 1329–1330.
11. World Health Organization. Information notes on HIV and COVID-19.2020. https://www.who.int/health-topics/HIV-AIDS
12. UNAIDS, G. H. AIDS statistics—2019 fact sheet, 2019.
13. McCormick, T. R. Principles of Bioethics. Ethics in Medicine University of Washington, 2013. https://depts.washington.edu/bioethx/tools/princpl.html
14. Centre for the Right to Health. HIV/AIDS and Human Rights in Nigeria. 2003
15. Ajuwon, A. J. Ethical issues in HIV/AIDS research, counseling and testing in Nigeria. Afr J Med Med Sci. 2017; 35 (Suppl.) 137–141.
16. Constitution of the Federal Republic of Nigeria, 1999.
17. Child's Rights Act, 2003
18. HIV and AIDS (Anti-Discrimination). Act, 2014.
19. Patients' Bill of Rights (PBoR), 2018.
20. Medical and Dental Practitioners' Act, 1990.
21. Code of Medical Ethics in Nigeria.
22. Legal Environment Assessment for HIV Response in Nigeria APRIL 22, 2016. Legal Environment Assessment for HIV Response in Nigeria. United Nations Development Programme (www.undp.org)

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Original Article **Open Access**

Post-exposure prophylaxis for HIV: A 10-year review of data from a tertiary health facility in northcentral Nigeria

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Abstract:

Background: Post-exposure prophylaxis (PEP) for human immunodeficiency virus (HIV) is the use of short-term antiretroviral therapy (ART) following a single risk exposure to a potential source of HIV infection. If commenced within 72 hours following exposure, PEP has been reported to be very effective in preventing replication and spread of the virus and therefore prevent acquisition of infection. PEP is recommended for exposures occurring in both occupational and non-occupational settings. The objectives of this study are to review the profile of patients and determine the reasons for accessing PEP services in our facility with a view to recommending evidence-based solutions and ultimately contributing to achieving zero transmission of HIV.
Methodology: A retrospective review of records of patients who received PEP for HIV in our facility over a 10-year period was carried out. Demographic and clinical variables of interest were extracted from the medical records and the PEP register of 252 eligible patients. Data were presented as frequencies, means, percentages and range. Bivariate analysis to determine association of clinical and demographic variables was carried out using the Statistical Package for the Social Sciences (SPSS) with $p<0.05$ considered as statistical significance.
Results: The mean age of the 252 patients studied was 26.25±11.81 years, and females accounted for 52.7%. The commonest reason for seeking HIV PEP was occupational exposure from sharps or needle sticks or splashes in 43.3% (109/252), while rape/sexual assault was the most common non-occupational reason for PEP in 29.0% (73/252) cases. Most (72.6% and 95.2%) of the patients presented within 24 hours and 72 hours respectively following exposure. While females accounted for 98.6% of cases of rape and sexual assault, children aged 10 years and below made up 28.8%.
Conclusion: Although most patients sought PEP for HIV due to occupational exposure, majority of those who came for non-occupational exposure were due to rape or sexual assault, most of which occurred in children and adolescents. There is need to institute measures aimed at reducing the menace of rape and sexual assault especially of minors in our society and for health facilities to have psychosocial support mechanisms for these patients.

Keywords: post-exposure; prophylaxis; HIV; retrospective; record

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Prophylaxie post-exposition au VIH: Examen sur 10 ans des données d'un établissement de santé tertiaire du centre-nord du Nigeria

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Résumé:

Contexte: La prophylaxie post-exposition (PPE) contre le virus de l'immunodéficience humaine (VIH) consiste en l'utilisation d'un traitement antirétroviral (TAR) à court terme après une exposition à un risque unique à une source potentielle d'infection par le VIH. Si elle est débutée dans les 72 heures suivant l'exposition, la PPE s'est avérée très efficace pour prévenir la réplication et la propagation du virus et donc empêcher l'acquisition de l'infection. La PEP est recommandée pour les expositions survenant dans des contextes professionnels et non professionnels. Les objectifs de cette étude sont d'examiner le profil des patients et de déterminer les raisons d'accéder aux services de PEP dans notre établissement en vue de recommander des solutions fondées sur des preuves et, à terme, contribuer à atteindre zéro transmission du VIH.
Méthodologie: Une revue rétrospective des dossiers de patients ayant reçu une PPE pour le VIH dans notre établissement sur une période de 10 ans a été réalisée. Les variables démographiques et cliniques d'intérêt ont été extraites des dossiers médicaux et du registre PEP de 252 patients éligibles. Les données ont été présentées sous forme de fréquences, moyennes, pourcentages et étendues. Une analyse bivariée visant à déterminer l'association de variables cliniques et démographiques a été réalisée à l'aide du progiciel statistique pour les sciences sociales (SPSS), avec $p<0,05$ considéré comme statistiquement significatif.
Résultats: L'âge moyen des 252 patients étudiés était de 26,25±11,81 ans et les femmes représentaient 52,7%. La raison la plus courante pour demander une PPE contre le VIH était l'exposition professionnelle à des objets tranchants, des piqûres d'aiguilles ou des éclaboussures dans 43,3% (109/252), tandis que le viol/l'agression sexuelle était la raison non professionnelle la plus courante pour la PPE dans 29,0% (73/252) des cas. La plupart (72,6% et 95,2%) des patients se sont présentés respectivement dans les 24 heures et 72 heures suivant l'exposition. Alors que les femmes représentaient 98,6% des cas de viol et d'agression sexuelle, les enfants âgés de 10 ans et moins en représentaient 28,8%.
Conclusion: Bien que la plupart des patients aient demandé une PPE pour le VIH en raison d'une exposition professionnelle, la majorité de ceux qui sont venus pour une exposition non professionnelle étaient dus à un viol ou à une agression sexuelle, dont la plupart se sont produits chez des enfants et des adolescents. Il est nécessaire d'instituer des mesures visant à réduire la menace de viol et d'agression sexuelle, en particulier contre les mineurs dans notre société, et de doter les établissements de santé de mécanismes de soutien psychosocial pour ces patients.

Mots clés: post-exposition; prophylaxie; VIH; rétrospective; enregistrer

Introduction:

The United Nations Program on AIDS (UNAIDS) reports that an estimated 1.5 million new infections with the human immunodeficiency virus (HIV) occurred in 2021 (1,2). Although there has been a decline in new infections, the UNAIDS had set targets to achieve a reduction of new HIV infections to 200,000 annually by 2030 (3). One of the strategies to reduce new HIV infections is the provision of postexposure prophylaxis (PEP) (3,4). Post-exposure prophylaxis (PEP) is the use of short-term antiretroviral therapy following a single risk exposure to a potential source of HIV with the aim of preventing acquisition of infection (5-8).

Post-exposure prophylaxis offers a window of opportunity to protect the exposed individual from acquiring HIV when commenced within 24 hours and not later than 72 hours following the exposure incident. Several studies have reported the efficacy of PEP when taken appropriately (6,7,9), and is recommended for exposures to sources of potential HIV infection occurring in both occupational and non-occupational settings. Occupational exposures occur mostly during provision of health care services and can result from percutaneous injury from contaminated needles or sharps or splashes of blood or other body fluids on mucosal surfaces or non-intact skin (10, 11). Studies have reported that about 65% of health care workers experience accidental sharp injuries during their career and 32% annually. This exposure places them at risk of infections with blood borne viruses, including HIV (12). Percutaneous injuries are reported to result in about 4.4% new HIV infections annually among health care workers (13)

Non-occupational exposures that require PEP include non-use or breakage of condom during sexual encounter with a partner whose HIV status is unknown or a virally unsuppressed HIV positive partner, sexual assault or rape as well as bites and other injuries outside occupational work environment (10, 11). Sexual assault and rape have been reported to occur frequently. In the United States (US), a study reported that 21.3% of women and 1.4% of men have reported a sexual assault or rape in their lifetime (14). Sexual contact remains a major contributor to the spread of HIV transmission globally (13).

Post-exposure prophylaxis for HIV requires the use of an appropriate antiretroviral drug (ARV) regimen that is determined after clinical assessment of the exposed individual and the exposure incident. A baseline assessment of the patient includes a history of the type of exposure, the HIV status of the source, and if positive, whether virally suppressed. The exposed individual's HIV test is also carried out to ascertain they are negative before commencing PEP (4,10,11,15). The ARV regimens for PEP evolved over time from sin-

gle drug in the early phase of HIV epidemic to two or three drug regimens due to the efficacy of the drugs and the development of resistance (16). PEP regimens are taken for 28 days during which the patient has to adhere to the medications and avoid other conditions that may render the PEP ineffective and thus lead to transmission of infection. These conditions include high risk behaviours that can expose them to HIV infection such as unsafe sex, sharing of needles as well as avoiding taking other medications that can lead to drug interactions with the ARVs (14,18).

When taken appropriately, PEP has been reported to prevent transmission of infection in up to 80% of exposures (6,17). Reports show that factors contributing to transmission of infection after PEP include non-adherence to medications, concomitant use of other medication that may interact with the ARVs, engaging in high-risk behaviour during PEP and follow-up period and possible exposure to a resistant strain of the virus (17). Some studies report that discussing the risks of PEP with the patient during clinical assessment and counselling helps to reduce failure of PEP (8,9). The risks aside from failure of PEP leading to transmission of infection also includes drug side effects (5-8).

While some data are now available on post-exposure prophylaxis globally, there is still a paucity of data from resource constrained settings on the practice and efficacy of PEP. This study seeks to add to the knowledge of service providers in our region with a view to improving existing practices. The aim of our study is to review the profile of patients and determine the reasons for accessing PEP services in our facility with a view to recommend ways of improving our services and therefore reduce the number of new HIV infections following accidental exposure.

Materials and method:

Study setting:

This study was carried out at the Special Treatment Clinic (STC) of Dalhatu Araf Specialist Hospital (DASH), Lafia, Nasarawa State, North-central, Nigeria. The hospital is a 450-bed tertiary health facility which provides all levels of healthcare services. The STC provides comprehensive ART as well as Sexually Transmitted Infections (STI) services.

The clinic receives cases of accidental exposures referred by other service providers from within and outside the facility. The services provided to patients including the antiretroviral (ARV) regimens provided for PEP are in line with the national guidelines (18).

Study design, population and method of sampling:

The study is a retrospective review which utilised secondary of patients of all ages who presented for post-exposure prophylaxis for HIV in the facility over 10 years (March 2013 to February 2023). All patients who had complete data of the desired variables were included in the study. A total of 335 patients had PEP during the period studied, but 103 of them had incomplete data and were therefore not included in the final analysis. The study was carried out between June 2023 to August 2023.

Data collection:

Data were collected from the post-exposure Prophylaxis (PEP) register and the patient's medical records folders. The PEP register has provision for collection of patients' information including the name, hospital and service identification numbers, age, sex, type of exposure, ART regimen, pre and post PEP HIV test, date PEP was commenced, and outcome of intervention. For this study, only data available and complete were extracted into a designed proforma.

The data include patient's identification (which was coded for confidentiality), age (to the nearest completed years), gender (male and female), type of exposure (occupational needle stick or blood slashes, sexual assault/ rape, non-occupational parenteral exposure (blades or bites), and unprotected sex or condom breakage), type of ARV regimen [Zidovudine (AZT), Lamivudine (3TC) and Efavirenz (EFV); or Tenofovir (TDF) and 3TC; or TDF, 3TC and EFV; or TDF, 3TC and Atazanavir/ ritonavir (ATV/r); or AZT, 3TC and Lopinavir/ ritonavir (LPV/r); or TDF, 3TC and Dolutegravir (DTG)].

Other information extracted from the patient's medical record folders include occupation of the patient, duration between exposure and presentation for PEP, HIV status of the source of exposure (negative, positive or unknown), and outcome of exposure at 1, 3 or 6 months (documented HIV test result or not documented).

Data analysis:

Data were entered into the Statistical Package for Social Science (SPSS) version 22 (SPSS Inc. Chicago, Illinois, USA). Recategorization of some variables including age group (1-5, 6-10 years etc) and duration of exposure before PEP (24 hours or less, 24-72 hours, and >72 hours) were done. Descriptive statistics of variables was carried out and presented as frequencies, means, percentages and range. Bivariate analysis to determine association of clinical and demographic variables was carried out with $p<0.05$ considered as statistical significance.

Ethical consideration:

Ethical approval for the study was obtained from the DASH Research Ethics Commi-

ttee. All patients' data were coded and personal identifiers removed. The PEP register and patients' folders were handled confidentially while extracting data by storing in locked cabinet when not in use. Soft copy of research data was stored in a password protected laptop accessible only to the researchers. Informed consent was not necessary for this study as the research did not involve contact with the patients.

Results:

A total of 335 patients were seen in the facility for PEP for HIV within the ten years period reviewed. However, only 252 had complete records and were included in the analysis. One hundred and thirty-two (52.4%) of the study subjects were females, with male to female ratio of 1:1.1. The age range of the patients was 1 and 55 years with a mean age of 26.25±11.807 years. Children aged 5 years or younger and those under 15 years constituted 7.5% (19/252) and 27.0% (68/252) respectively.

Majority (64.6%) of the study population were in the age range 21-40 years, while 53.1% were in the age range 21-35 years (Table 1). Medical doctors (16.3%), children (14.3%), nurses (12.7%) and civil servants (6.7%) were the major occupations. A total of 42.1% (106/252) patients were in health-related occupations while 57.9% (146/252) were either in non-health related occupations, children or unemployed (Table 1).

Table 1: Frequency distribution of the demographic characteristics of patients who had HIV post-exposure prophylaxis at Dalhatu Araf Specialist Hospital (DASH), Lafia, Nigeria

Demographic variables	Number	Percentage
Gender		
Male	120	47.6
Female	132	52.4
Age group (years)		
1-5	19	7.5
6-10	15	6.0
11-15	12	4.8
16-20	22	8.7
21-25	41	16.3
26-30	57	22.6
31-35	36	14.3
36-40	29	11.5
41-45	8	3.5
46-50	9	3.6
51-55	4	1.6
Mean age (26.25±11.81 years)		
Occupation		
Army	1	0.4
Artisan	3	1.2
Ward attendants	7	2.8
Banker	3	1.2
Business	9	3.6
CHEW	4	1.6
Child	36	14.3
Civil servant	17	6.7
Youth Corper	3	1.2
Medical Doctor	41	16.3
Driver	3	1.2
Engineer	1	0.4
Housewife	7	2.8
Journalist	1	0.4
Laboratory scientist	5	2.0
Laboratory technician	8	3.2
Laborer	1	0.4
Nurse	32	12.7
Police	2	0.8
Student	48	19.0
Student nurse	9	3.6
Trader	7	2.8
Unemployed	4	1.6
Type of occupation		
Health-related	106	42.1
Non-health related & unemployed	146	57.9

Table 2: Frequency distribution of the clinical characteristics of patients who had HIV post-exposure prophylaxis at Dalhatu Araf Specialist Hospital (DASH), Lafia, Nigeria

Clinical variables	Number	Percentage
Type of exposure		
Occupational-Needle stick/sharps injuries/splashes	109	43.3
Rape or sexual assault	73	29.0
Bites/blades/sharps in non-occupational setting	17	6.7
Unprotected sex/condom breakage	53	21.0
Time duration from exposure to PEP		
24 hours or less	183	72.6
24 -72 hours	57	22.6
More than 72 hours	12	4.8
Baseline HIV test of patient		
Negative -recorded	216	85.7
Not recorded	33	13.1
Declined testing	3	1.2
HIV status of source of exposure		
Negative	10	4.0
Positive	112	44.4
Unknown	130	51.6
ARV regimen given		
AZT+3TC+EFV	15	6.0
TDF+3TC+EFV	37	14.7
TDF+3TC+ DTG	89	35.3
TDF+3TC	4	1.6
TDF+3TC+ATV/r	90	35.7
TDF+3TC+LPV/r	17	6.7

AZT- Zidovudine; 3TC- Lamivudine; TDF – Tenofovir; DTG- Dolutegravir; ATV- Atazanavir; LPV- Lopinavir; r- ritonavir

Occupational exposure due to accidental needle stick/sharps injury or blood splash were the most frequent reason for seeking PEP with 109 (43.3%) accounting for it, while rape/sexual assault was the most common non-occupational reason, accounting for 29% (79/252), followed by unprotected sex or condom breakage in 53 (21.0%) cases (Table 2). The time duration from exposure incident to presentation for PEP ranged between 2 and 312 hours, with a mean time of 27.62 ± 38.56 hours.

Majority (72.6%) presented within the first 24 hours and most (95.2%) within 72 hours following exposure. The HIV status of the source of exposure was unknown in 130 (51.6%) cases, positive in 112 (44.4%) while 10 (4.0%) were HIV negative (Table 2). Two hundred and sixteen (85.7%) of the patients had recorded baseline HIV test and all were negative. Three (1.9%) participants declined testing and 33 (13.1%) had no baseline HIV test recorded. The most common ARV regimen given to the patients were TDF+3TC and ATV/r and TDF+3TC and DTG in 90 (35.7%) and 89 (35.3%) patients respectively (Table 2).

As shown in Table 3, 52.5% of males and 34.8% of females had occupational exposure (OR=2.066; 95% CI:1.25-3.43; p=0.005). Rape and sexual assault occurred in 55.4% of females and 0.8% of males (OR=0.007; 95% CI: 0.0001-0.05; p<0.0001). Unprotected sex and condom breakage occurred in 38.3% of males and 5.3% females (OR=11.1; 95% CI: 4.78-25.86; p<0.0001).

Compared with other age groups, patients in the age groups 26-30, 31-35, and 36-40 years had significantly higher risk of occupational sharps exposure (x^2=40.658; p< 0.0001), while those in the age groups 1-5, 6-10, 11-15 and 16-20 years had significantly higher risk of rape/sexual assault (x^2=79.561, p< 0.0001). Similarly, patients in the age groups 1-5 and 6-10 years, compared with other age groups, had significantly higher risk of non-occupational sharps exposure (x^2=38.17, p< 0.0001), while patients in age group 36-55 years had significantly higher risk of unprotected sex (x^2=24.303, p=0.0068).

About 93.4% of patients with health-related occupations had occupational sharps/ splashes HIV PEP exposure, and this was significantly higher than 6.9% in those with non-health related occupations/unemployed (OR= 192.34, p<0.0001). About 1.9%, 0.9% and 3.8% of patients with health-related occupation respectively had rape/sexual assault, non-occupational sharps injury, and unprotected sex HIV PEP exposures, compared to 48.6%, 10.4% and 33.6% respectively in those with non-health-related occupations/unemployed.

Patients with health-related occupations, compared to those with non-health related occupations/unemployed, had significantly higher risk of HIV PEP exposure through occupational sharps injury and blood splashes (OR=192.34; 95% CI: 70.74-522.96; p < 0.0001), and significantly lower risk of rape/ sexual assault (OR=0.02; 95% CI: 0.005-0.085; p< 0.0001), non-occupational sharps injury (OR= 0.077, 95% CI: 0.01-0.59; p= 0.0015) and unprotected sex (OR=0.078; 95% CI: 0.027-0.223; p< 0.0001).

Table 3: Bivariate analysis of demographic characteristics of patients and type of exposure/reasons for accessing HIV post-exposure prophylaxis

Demographic variables	Type of exposure			
	Occupational-needle stick/sharps/splashes of body fluids (n=109, 43.3%)	**Rape or sexual assault (n=73, 28.9%)**	**Bites/sharps in non-occupational setting (n=17, 6.7%)**	**Unprotected sex or condom breakage (n=53, 21.0%)**
Gender				
Male	63 (52.5)$^{+}$	1 (0.8)$^{++}$	10 (8.3)	46 (38.3)$^{+}$
Female	46 (34.8)	72 (55.4)	7 (5.3)	7 (5.3)
OR (95% CI)	2.066 (1.25-3.43)	0.007 (0.0001-0.05)	1.623 (0.59-4.41)	11.1 (4.78-25.86)
p	0.0052*	<0.0001*	0.452	<0.0001*
Age group (years)				
1-5	2 (10.5)	10 (52.6)$^{+}$	7 (36.8)$^{+}$	0
6-10	1 (6.7)	11 (73.3)$^{+}$	3 (20.0)$^{+}$	0
11-15	1 (8.3)	11 (91.7)$^{+}$	0	0
16-20	5 (22.7)	13 (59.1)$^{+}$	2 (9.1)	2 (9.1)
21-25	17 (41.5)	13 (31.7)	1 (2.4)	10 (24.4)
26-30	34 (59.6)$^{+}$	10 (17.5)	1 (1.8)	12 (21.1)
31-35	23 (63.9)$^{+}$	0	2 (5.6)	11 (30.6)$^{+}$
36-40	16 (55.2)$^{+}$	3 (10.3)	1 (3.4)	9 (31.0)$^{+}$
41-45	4 (50.0)	1 (12.5)	0	3 (37.5)$^{+}$
46-50	4 (44.4)	1 (11.1)	0	4 (44.4)$^{+}$
51-55	2 (50.0)	0	0	2 (50.0)$^{+}$
x^2	40.658	79.561	38.173	24.303
p	<0.0001*	<0.0001*	<0.0001*	0.0068*
Types of occupation				
Health-related	99 (93.4)$^{+}$	2 (1.9)$^{++}$	1 (0.9)$^{++}$	4 (3.8)$^{++}$
Non-health-related and unemployed	10 (6.9)	71 (48.6)	16 (10.9)	49 (33.6)
OR (95% CI)	192.34 (70.74-522.96)	0.020 (0.005-0.085)	0.077 (0.010-0.59)	0.078 (0.027-0.223)
p	<0.0001*	<0.0001*	0.0015*	<0.0001*

X^2 - Chi square; OR – Odds Ratio; CI – Confidence Interval; n - number; % - percentage; * - statistically significant at $p<0.05$; $^{+}$ - significantly higher; $^{++}$ - significantly lower

Table 4 shows the distribution of patients according to the time duration from exposure to presentation for PEP and it shows that there was no difference in time of presentation with 24 hours of exposure between the gender as 72.5% of the males and 72.7% of the females presented after 24 hours of exposure (OR=0.987; 95% CI: 0.568-1.721; p=1.000), but less number of males (1.7%) than females (7.6%) presented after 72 hours of exposure (OR=0.21; 95% CI:0.044-0.964; p=0.037).

Majority of the patients aged 1-5 years (84.2%), 31-35 (80.6%) and all those aged 51-55 years (100.0%) presented within the first 24 hours for PEP (x^2= 29.357, p=0.0011), while the number of those aged 6-10 years (33.3%) who presented after 72 hours of exposure was significantly higher than those in other age groups (x^2=18.698, p=0.0022).

Among the occupational groups, most of the medical doctors (97.6%) and all the laboratory scientists and technicians (100%, n=7) presented within 24 hours of exposure. Among patients who had occupational exposure, 88.1% presented within 24 hours and 99.1% within 72 hours, while 61.6% of the rape/sexual assault cases presented within 24 hours of exposure and 12.3% presented after 72 hours. Among the 112 patients who were exposed to HIV positive source, 90 (80.4%) and 101 (99.2%) presented within 24 hours and 72 hours respectively after exposure, while 86 (63.8%) of those exposed to a source with unknown HIV status presented within 24 hours and 11 (8.5%) presented after 72 hrs.

Significantly higher number of patients with health-related occupations (88.7%) presented within 24 hours of exposure compared to patients with non-health-related occupations (60.9%) (OR=5.02; 95% CI: 2.52-9.97; p<0.0001), while significantly lower number presented within 24-72 hours (OR= 0.29; 95% CI: 0.14-0.58; p=0.0002) and after 72 hours post-exposure (OR=0.05; 95% CI: 0.003-0.86; p=0.0016). Significantly higher number of patients with occupational sharps (88.1%) and non-occupational sharps/ bites (88.2%) exposures presented within 24 hours (x^2=32.123, p <0.0001), while significantly high number of patients with unprotected sex/condom break exposure (45.3%) presented within 24-72 hours (x^2=25.557, p< 0.0001), and significantly high number of rape /sexual assault exposure patients presented after 72 hours exposure (x^2=13.733, p=0.003).

Only 14 (5.6%) patients had documented outcome at least once at 1, 3 or 6 months after PEP, with all the documented results being HIV negative while, 94.4% had no documented follow-up or outcome in this study.

Table 4: Bivariate analysis of demographic and clinical characteristics of patients and duration of exposure before presenting for HIV post-exposure prophylaxis

Demographic variables	Duration of exposure before presenting for PEP		
	Within 24 hours (n=183, 72.6%)	**24-72 hours (n=57, 22.6%)**	**More than 72 hours (n=12, 4.8%)**
Gender			
Male	87 (72.5)	31 (25.8)	2 (1.7)$^{++}$
Female	96 (72.7)	26 (19.7)	10 (7.6)
OR (95% CI)	0.987 (0.568-1.721)	1.42 (0.79-2.57)	0.21 (0.044-0.964)
p	1.000	0.2917	0.037*
Age group (years)			
1-5	16 (84.2)$^{+}$	1 (5.3)	2 (10)
6-10	8 (53.3)	2 (13.3)	5 (33.3)$^{+}$
11-15	7 (58.3)	4 (33.3)	1 (8.3)
16-20	7 (68.2)	4 (27.3)	1 (4.5)
21-25	31 (75.6)	8 (19.5)	2 (4.9)
26-30	43 (75.4)	13 (22.8)	1 (1.8)
31-35	29 (80.6)$^{+}$	7 (19.4)	0
36-40	22 (75.9)	7 (24.1)	0
41-45	4 (50.0)	4 (50)	0
46-50	4 (44.4)	5 (55.6)	0
51-55	4 (100.0)$^{+}$	0	0
x^2	29.357	14.698	18.698
p	0.0011*	0.0996	0.0022*
Type of occupation			
Health-related	94 (88.7)$^{+}$	12 (11.3)$^{++}$	0^{++}
Non-health-related & unemployed	89 (60.9)	45 (30.8)	12 (8.2)
OR (95% CI)	5.02 (2.52-9.97)	0.29 (0.14-0.58)	0.05 (0.003-0.86)
p	<0.0001*	0.0002*	0.0016*
Type of exposure			
Occupational-needle/sharps/splashes	96 (88.1)$^{+}$	12 (11.0)	1 (0.9)
Rape or sexual assault	45 (61.6)	19 (26.0)	9 (12.3)$^{+}$
Non-occupational sharps/bites	15 (88.2)$^{+}$	2 (11.8)	0
Unprotected sex or condom breakage	27 (50.9)	24 (45.3)$^{+}$	2 (3.8)
x^2	32.123	25.577	13.733
p	<0.0001*	<0.0001*	0.0033*
HIV status of source of exposure			
Negative	10 (100.0)$^{+}$	0	0
Positive	90 (80.4)	21 (18.8)	1 (0.9)
Unknown	83 (63.8)	36 (27.7)	11 (8.5)$^{+}$
x^2	12.175	5.793	8.120
p	0.0023*	0.0552	0.0172*

x^2 - Chi square; OR – Odds Ratio; CI - Confidence Interval; n – number; % - percentage; * - statistically significant at $p<0.05$; $^{+}$ - significantly higher; $^{++}$ - significantly lower

Discussion:

The results of this study showed that people who access services in this facility are aware of the availability of PEP for HIV and are utilizing the service in a timely manner as indicated by the findings that majority 95.2% (240/252) of the patients who came for PEP did so within 72 hours following exposure. This finding is similar to a report from southeast Nigeria which found that half of the patients that reported for PEP did so within 24hours and most within 72 hours (19). These findings are encouraging and show that most people are aware of and seek PEP within the ideal time frame of 72 hours that PEP will be effective in preventing the acquisition of HIV following a single incident of exposure (4-6,10). The guidelines recommend that PEP should commence as soon as possible after exposure and not later than 72 hours for it to be effective. However, factors such as adherence to the medications and other factors such as avoiding drug-drug interactions must be observed for PEP to be effective (4-7,10,11).

This study found that more females (52.4%) than males (47.6%) presented for PEP, similar to other reports from Nigeria (18-21). However, Kuoanfack et al., (22) in 2019 reported from their study in Cameroon, West Africa that 70% of those who sought PEP in their study were males. The preponderance of the female gender in most of these reports may be due to the health-seeking behavior that has generally been shown to be higher in females. However, it may also be due to the fact that more females are victims of the risks that lead to potential exposure to HIV such as sexual assault and rape.

Majority (64.6%) of the patients in this study were aged between 21-40 years. Several reports found a preponderance of this age group in those seeking for PEP (20,21, 23,24). The most common reason for seeking PEP in the study was occupational exposure through needle stick or sharps or splashes from body fluids accounting for 43.3% (109/ 252). Rape or sexual assault accounted for the second commonest reason and the commonest non-occupational reason for PEP. These findings are similar to the report by Oyedum et al., (20) from southeast Nigeria. However,

it is in contrast to several reports that found rape and sexual assaults to be the commonest reason for seeking PEP (19,21,23,24). The lower number of rape and sexual assault compared to occupational exposure in our study may be due to the lack of reporting or the effects of stigma attached to rape thus leading to refusal to access care or report to authorities. Also, many healthcare workers in the faculty have knowledge of PEP following sensitization and display of posters on how to manage accidental exposures to potential source of blood-borne viruses by the hospital Infection Prevention and Control Committee (IPCC).

Majority (98.6%) of those who sought for PEP on account of rape or sexual assault in this study were females, which constituted 55.4% (72/132) of all the female patients (p<0.0001), and 75.3% of the rape/sexual assault occurred in children between 1 to 15 years and adolescents 16-20 years of age. Similar findings have been reported in studies from Nigeria and West Africa (19,22). The high number of children aged five years or younger who accessed PEP due to rape/sexual assault during the period of this study (13.7%, 10/73) is very disturbing, as this actually constituted 52.4% of exposure within that age group, and 43.9% (32/73) in children 15 years or younger. Reports from South Africa found that 15.8% of people accessing PEP for sexual assault were 10 years or younger (25). Another study from the United States reported that 12.7% of those exposed to rape or sexual assault were aged 10 years or younger and 30% were between 11 and 17 years of age (14). There is paucity of accurate data from Nigeria on rape and sexual assault and therefore, figures being reported in most studies including ours may be an underestimation mainly due to the under-reporting of reporting of rape and sexual assault. Many factors account for the lack of reporting including cultural, stigma and the apparent perception of lack of prosecution of perpetrators (26).

Most of those who sought HIV PEP for occupational reasons were those in health-related occupations such as doctors, nurses and laboratory workers. Occupational hazards especially needles stick and sharps injuries are common among HCWs who provide direct patient care including junior doctors and nurses. Most of these exposures occur in emergency settings (5,7,8). This study found that majority of the cases in rape/sexual assault were in non-health related occupations (students) and children. This finding is similar to reports from other studies from Nigeria and West Africa (19, 22, 24).

Unprotected sex or condom breakage accounted for the reason for PEP in 21% (53/252) of cases with males accounting for 86.6% (46/53), constituting 38.3% of exposure source for males compared to 5.3% for the females (p< 0.0001). This finding in similar to the report from West Africa where unprotected sex and condom breakage accounted for about 25% of cases seeking PEP (22). The high number of males compared to females engaging in unprotected sex or experiencing condom breakage may be due to the risk-taking behavior mostly associated with the male gender. Many of the sexual encounters are with a partner whose HIV serological status is unknown which might indicate casual sex. This study found that the HIV status of the source of exposure was unknown in 51.6% (130/252) of cases and only 44.4% were exposed to a known HIV positive source. Although a lot of information is available on the risks for acquiring HIV, a lot more needs to be done to educate people on risk reduction if the target of achieving HIV epidemic control is to be achieved.

Our study found significant associations between early presentation for HIV PEP (within 24 hours) and patients with health-related occupations, certain age groups (1-5, 31-35 and 51-55 years), occupational sharps exposure, and exposure to an HIV positive or negative source. While most of those with occupational and non-occupational sharps/bites presented within 24 hours, significantly high number of those with unprotected sex/condom break exposure presented within 24-72 hours, and significantly high number of those with rape/sexual assault exposure presented after 72 hours exposure. These findings are like those reported by Nwolisa et al., (21). Some factors that have been reported to influence access to PEP include awareness, level of education, knowledge of HIV status of source and female gender (4,16,19).

This study only recorded outcome in 5.6% (14/252) of the patients studied. Similar findings were reported in other studies where most patients who had PEP did not return for follow up with their outcome unknown (19, 20). The guideline for PEP recommends that follow up HIV serological test be carried out at one, three and six months after completion of the 28-day PEP regimen (4-8). However, as observed in this study, many patients do not return for follow up. Although patients need proper counselling before the commencement of PEP, this is often not the case as PEP is often commenced as an emergency. However, there is need to institute and carry out proper counselling of all patients who are placed on PEP to ensure adherence to their medications and also to complete the follow up for PEP including the HIV serological testing at baseline and at 1, 3 and 6 months after completion of the PEP ARV regimen. This will help improve the documentation and provide data on the rate of seroconversion following PEP if it does occur.

Conclusion:

This study has brought to the fore the need to put in place societal measures to reduce the rate of rape and sexual assault especially on children. There is also the need to ensure victims have access to PEP services as well as psychosocial and legal support as soon as possible. This will encourage improved reporting especially for victims of rape, thus reducing their risk of acquiring HIV infection. There is also need to institute measures to improve patients follow up and documentation of outcomes. This will help in the assessment of the success or otherwise of the post-exposure prophylaxis measures.

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Contributions of authors:

AES and BSO developed the study concept and design; AES, AAA, and AA were involved in data collection and entry; AES and BSO performed data analysis; AES, AAA, BSO, BIA, MM, AA and AMC contributed to the result interpretation and manuscript preparation. All authors approved the final manuscript.

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No conflict of interest is declared

References:

1. Global HIV and AIDS statistics - Fact Sheet. www.unaids.org/en/resources (Accessed 9/7/23)
2. The Global Health Observatory. www.who.int (Accessed 9/7/23).
3. Understanding Fastrack. Accelerating action to end AIDS Epidemic by 2030. www.unaids.org/files (Accessed 9/7/24)
4. Post-exposure prophylaxis. www.hiv.gov/hiv (Accessed 9/7/23).
5. Post-exposure prophylaxis (PEP) for HIV prevention. www.betterhealth.vic.gov.au (Accessed 6/7/23).
6. Post-exposure prophylaxis (PEP). www.cdc.gov/hiv/risk/PEP (Accessed 8/7/23).
7. Post-exposure prophylaxis (PEP). www.aidsmap.com (Accessed 9/7/23).
8. Sultan, B., Nenn, P., and Waters, L. Current perspectives in HIV Post-exposure prophylaxis. HIV AIDS (Auck). 2014;6: 147. doi:10.2147/HIV.S46585
9. Heendeniya, A., and Bogoch, I. HIV prevention with post-exposure prophylaxis-in-pocket. Lancet 2019; (10E): 494. https://doi.org/10.1016/S2468-3677(19)40152-5
10. PEP. HIV Basics. www.cdc.gov/hiv/workplace
11. HIV and Occupational exposure. www.cdc.gov/hiv/workplace (Accessed 9/7/2023)
12. Mengistu, D. A., Tolera, S. T., and Demmu, Y. M. Worldwide prevalence of occupational exposure to needle stick injury among healthcare workers: A systematic review and meta-analysis. Can J Infect Dis Med Microbiol. 2021; 9019534. doi: 10.1255/2021/9019534
13. Draughan, J. E. Sexual Assault injuries and increased risk of HIV transmission. Adv Emerg Nurs J. 2012; 34 (1): 82-87. doi.10.1097/TME.0b013e3182439e1a
14. Dehaan, E., McGowan, J. P., Fine, S. M., et al. PEP to prevent HIV infection. HIV Clinical guidelines program 2000 – 2020. www.ncbi.nlm.nih.gov/books.
15. Post-exposure HIV prophylaxis. BMJ best practice Oxford academic. https://academic.oup.com>article (Accessed 8/7/23).
16. Owolabi, R. S., Alabi, P., Ajayi, S., et al. Knowledge and practice of post-exposure prophylaxis (PEP) against HIV infection among Health care providers in a Tertiary Hospital in Nigeria. J Int Assoc Phys AIDS Care. 2012; 11(3): 179-183. https://doi.org/10.1177/1545109711401409.
17. Landovitz, R. J., and Currier, J. S. Post-exposure prophylaxis for HIV infection. N Engl J Med 2009; 361: 1768 -1775. doi:10.1056/NEJMcp0904189
18. National guidelines for HIV prevention, treatment and care. National AIDS and STIs Control Programme. Federal Ministry of Health Nigeria. https://www.nascp.gov.ng (Accessed 7/8/2023).
19. Iloanusi, S. H., Mgbere, O. O., Abughiosh, S. M., and Essien, E. J. HIV Non-occupational post-exposure prophylaxis in Nigeria: A systematic Review of Research evidence and practice. Int J MCH AIDS. 2019; 8 (2): 101-119. doi.10.21106/ijma.287 accessed 5/7/2023.
20. Oyedum, C. C., Chukwuka, C., Iyoke, C. A., and Omotola, O. F. HIV postexposure prophylaxis (PEP) in a Nigerian Tertiary Health Institution. J Int Assoc Phys AIDS Care. 2011; 10 (3): 171-175. doi:10.1177/1545109710397893
21. Nwolisa, E., Ikejiaku, U., Odinaka, K., et al. Reasons for seeking post-exposure prophylaxis for HIV in a Paediatric infectious disease clinic in Owerri, Nigeria. Niger Med Pract. 2021; 80: 1-6.
22. Kouanfack, C., Meli, H., Cumber, S. N., et al Non-occupational HIV post-exposure prophylaxis: A 10-year Retrospective Review of data following sexual exposure from Yaounde Central Hospital, Cameroon. Int J MCH AIDS. 2019; 8(2):138-145. doi.10.21106/ijma.311
23. Olowookere, S. A., and Fatiregun, A. A. Human Immunodeficiency Virus Postexposure prophylaxis at Ibadan, Nigeria. J Int Assoc Phys AIDS Care. 2010; 9(3): 187-190. doi:10.1177/1545109709359938
24. Ufornwa, E.C. and Okoroiwu, H.U. Causes of Initiation of postexposure prophylaxis in a tertiary health care institution in Southeastern Nigeria. Niger Hlth J. 2012; 21 (3): 149- 168.
25. Muriuki, E.M., Kimani, J., Machuki, Z., Kiarie, J., and Roxby, A. C. Sexual assault and HIV post-exposure prophylaxis at an urban African Hospital. AIDS Patient Care STDS. 2017; 31 (6): 255-260. doi.10.1089/apc.2016-0274.
26. Aborishade, R. A. Barriers to Rape Reporting for Nigerian Women: The case of female University students. Int J Criminol Sociol Theory. 2014; 7 (2): 1-14.

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Original Article **Open Access**

Prevalence of asymptomatic significant bacteriuria and antibiotic susceptibility pattern of bacterial isolates in HIV-infected patients in Ilorin, Nigeria

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Abstract:

Background: Urinary tract infection (UTI) is one of the most common type of infections worldwide, and this is usually preceded by asymptomatic significant bacteriuria (ASB). The emergence of antibiotic resistance in bacteria responsible for UTI makes this entity of public challenge, which has been fueled by human immunodeficiency virus (HIV) infection. This study determined the prevalence of ASB and antimicrobial susceptibility pattern of bacteria isolated from urine samples of selected HIV-infected patients in Ilorin, Nigeria.
Methodology: A cross-sectional study of 300 randomly selected HIV-positive patients from Sobi Specialist and Civil Service hospitals in Ilorin, Kwara State, Nigeria, was conducted from January to March 2019. Clean-catch midstream urine samples were aseptically collected from each selected participant, cultured on CLED and Blood agar plates, and incubated aerobically at 37°C for 24 hours. The bacterial growth on the culture plates were identified using standard microbiological techniques. The Kirby-Bauer disk diffusion method was used to determine the antibiotic sensitivity of the bacterial isolates against a panel of antibiotics.
Results: The overall prevalence of ASB among the participants was 22.3%. *Staphylococcus aureus* (41.8%, 28/67), *Escherichia coli* (25.4%, 17/67), and *Klebsiella pneumoniae* (17.9%, 12/67) were the predominant bacterial isolates. *Staphylococcus aureus* was resistant to amoxicillin-clavulanate (64.3%), ceftriaxone (53.6%), ciprofloxacin (64.3%), and nalidixic acid (71.4%); *E. coli* was also resistant to amoxicillin-clavulanate (70.6%), ceftriaxone (53.6%), ciprofloxacin (52.9%) and nalidixic acid (64.7%); and *K. pneumoniae* was moderately resistant to amoxicillin-clavulanate (50.0%) and resistant to ciprofloxacin (58.3%) and nalidixic acid (75.0%). Multidrug resistance (MDR) was observed in 40.8% of the isolates.
Conclusion: The isolation rate of high MDR bacteria highlights the growing challenge of ASB and UTIs that are becoming increasingly difficult to treat with available antibiotics. Health professionals should be aware of regional resistance pattern to consider in the current empirical antimicrobial therapy for ASB and UTIs among HIV-infected patients. Strategies to mitigate spread of resistance are urgently needed in the study area.

Keywords: antimicrobial resistance; asymptomatic significant bacteriuria; prevalence; HIV

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Prévalence de la bactériurie significative asymptomatique et schéma de sensibilité aux antibiotiques des isolats bactériens chez les patients infectés par le VIH à Ilorin, au Nigeria

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Résumé:

Contexte: L'infection des voies urinaires (UTI) est l'un des types d'infections les plus courants dans le monde, et elle est généralement précédée d'une bactériurie significative asymptomatique (ASB). L'émergence de la résistance aux antibiotiques chez les bactéries responsables des infections urinaires fait de cette entité un défi public, qui a été alimenté par l'infection par le virus de l'immunodéficience humaine (VIH). Cette étude a déterminé la prévalence de l'ASB et le schéma de sensibilité aux antimicrobiens des bactéries isolées à partir d'échantillons d'urine de patients infectés par le VIH sélectionnés à Ilorin, au Nigeria.
Méthodologie: Une étude transversale de 300 patients séropositifs sélectionnés au hasard dans les hôpitaux spécialisés et de la fonction publique de Sobi à Ilorin, dans l'État de Kwara, au Nigéria, a été menée de janvier à mars 2019. Participant, cultivé sur des plaques de CLED et de gélose au sang, et incubé en aérobiose à 37°C pendant 24 heures. La croissance bactérienne sur les plaques de culture a été identifiée en utilisant des techniques microbiologiques standard. La méthode de diffusion sur disque Kirby-Bauer a été utilisée pour déterminer la sensibilité aux antibiotiques des isolats bactériens par rapport à un panel d'antibiotiques.
Résultats: La prévalence globale de l'ASB parmi les participants était de 22,3 %. *Staphylococcus aureus* (41,8%, 28/67), *Escherichia coli* (25,4%, 17/67) et *Klebsiella pneumoniae* (17,9%, 12/67) étaient les isolats bactériens prédominants. *Staphylococcus aureus* était résistant à l'amoxicilline-acide clavulanique (64,3%), à la ceftriaxone (53,6%), à la ciprofloxacine (64,3%) et à l'acide nalidixique (71,4%); *E. coli* était également résistant à l'amoxicilline-acide clavulanique (70,6%), à la ceftriaxone (53,6%), à la ciprofloxacine (52,9%) et à l'acide nalidixique (64,7 %); et *K. pneumoniae* était modérément résistant à l'amoxicilline-acide clavulanique (50,0%) et résistant à la ciprofloxacine (58,3%) et à l'acide nalidixique (75,0%). La multirésistance (MDR) a été observée dans 40,8% des isolats.
Conclusion: Le taux d'isolement des bactéries MDR élevées met en évidence le défi croissant des ASB et des infections urinaires qui deviennent de plus en plus difficiles à traiter avec les antibiotiques disponibles. Les professionnels de la santé doivent être conscients du schéma de résistance régional à prendre en compte dans la thérapie antimicrobienne empirique actuelle pour l'ASB et les infections urinaires chez les patients infectés par le VIH. Des stratégies pour atténuer la propagation de la résistance sont nécessaires de toute urgence dans la zone d'étude.

Mots-clés: résistance antimicrobienne; bactériurie importante asymptomatique; prévalence; VIH

Introduction:

Globally, an estimated 38.4 million people are living with human immunodeficiency virus (HIV) with a high number of infected people (~25.6 million) in sub-Saharan Africa (1,2). Annually, an estimated 40.1 million people died from AIDS-related illnesses (3). In people living with HIV/AIDS (PLWHA), almost every part of the genitourinary system is affected with different diseases (4). HIV infects vital cells in the human immune system such as $CD4^+$ (helper) T cells, macrophages and dendritic cells (5). HIV infection leads to low levels of $CD4^+$ T cells through three main mechanisms; direct viral killing of infected cells, increased rate of apoptosis in infected cells, and killing of infected $CD4^+$ T cells by $CD8^+$ (cytotoxic) T-lymphocytes that recognize infected cells. When $CD4^+$ cells count decline below a critical level, cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections (6).

In man, the urinary tract is the second commonest site after the respiratory tract for bacterial infection (7). The urinary tract is an anatomical unit and infection of one part could generally spread to other parts (8). When the infection is localized at such single sites as the kidneys, it is referred to as pyelonephritis, to the urethra as urethritis or restricted to the bladder as cystitis and to the prostate as proctitis. It affects both old and young leading to a number of deaths either from acute infection or from chronic renal failure (9). Specific group of people are at increased risk of urinary tract infection. Vulnerable populations are women, especially during pregnancy, infants and elderly patients (10). Also, certain conditions may increase susceptibility to infections i. e. spinal cord injuries, urinary catheters, diabetes, multiple sclerosis, immunodeficiency and underlying urologic abnormalities (11). UTI is one of the most

common bacterial infections causing morbidity and hospitalization in HIV-infected individuals (12).

HIV disease is associated with a variety of renal syndromes in patients with low $CD4^+$ cell counts associated with neurological complications which lead to urinary stasis and infection (13). Once the $CD4^+$ count falls below 200 cells/mm^3, the individual is at risk of a variety of opportunistic infections, from fungi, protozoa, viruses and bacteria. The most predominant causative organisms are encapsulated bacteria notably *Streptococcus pneumoniae* and *Haemophilus influenzae*, but non-typhoidal *Salmonella, Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been implicated (14). Among opportunistic infections, UTI accounts for approximately 60% of AIDS defining illnesses (7).

There are also reports of change in the resistance pattern over the last decade leading to serious therapeutic challenges (15). Since the distribution of these pathogens and their susceptibility to antibiotics varies regionally, and treatment for UTI is usually empirical, it is mandatory that there is an adequate knowledge of the epidemiological characteristics of the pathogens involved and their antibiotic susceptibility patterns. This will help to achieve good therapeutic outcomes and prevent the emergence of drug-resistant bacteria strains (16). This objective of this study is to determine the prevalence of ASB and the antimicrobial susceptibility pattern of bacteria isolated from urine samples of HIV-infected patients in selected HIV clinics in Ilorin, Nigeria.

Materials and method:

Study setting and participants:

This study was carried out in the HIV clinics of Sobi Specialist and Civil Service hospitals in Ilorin, the capital city of Kwara State, Nigeria, over a period of 3 months (January to March 2019). The laboratory aspect of the study was conducted at the department of microbiology, University of Ilorin Teaching Hospital, and the department of microbiology, Kwara State University, Malete.

A total of 300 HIV-infected individuals receiving HIV care were consecutively recruited over the period of the study, and the sample size was calculated using a formula as previously described (17).

Ethical consideration:

Ethical approval was obtained from the Ethics Review Committees of the Ministry of Health, Ilorin, and Kwara State University Ethics Committee. Written informed consent was obtained from the participants before inclusion in the study.

Data and sample collection:

Appropriately labeled universal sample bottle was given to each participant for collection of clean catch midstream urine specimen following explanation of the procedure for such collection. The samples were immediately transported to the department of microbiology, University of Ilorin Teaching Hospital for analysis.

Demographic and clinical information were obtained from each patient including the age and gender, history of UTI, antibiotic use, and history of opportunistic infections. Participants whose urine sample yielded growth were requested to provide another early morning clean-catch mid-stream urine sample for confirmation.

Microscopic examination and culture of urine:

Each urine sample was well mixed and centrifuged at 3200 rpm for 5 mins. The supernatant was decanted and a drop of the sediment was placed on a sterile glass slide and covered with a cover slip and was viewed under 10x and 40x objective lens to observe for white blood cells, red blood cells, casts, crystals, epithelial cells, and ova of parasites.

Each urine sample was shaken to allow for homogeneity. Using a standard sterile wire loop (calibrated to deliver 0.001ml of urine), a loopful of the urine was aseptically inoculated onto Blood and Cystine Lactose Electrolyte Deficient (CLED) agar plates. The plates were incubated aerobically at 37°C for 24 hours, and examined macroscopically and microscopically for bacterial growth (18).

Significant bacteriuria was determined on the culture plate when there was $\geq 10^5$ colony forming units per milliliter (19). Colonies of urine culture with significant bacteriuria were Gram stained and bacteria identified by conventional biochemical tests (20).

Antimicrobial susceptibility testing (AST):

Using the modified Kirby-Bauer disk diffusion test, 3 to 5 pure colonies of the isolates to be tested were selected, and emulsified in sterile saline solution, thoroughly mixed to prepare bacterial inoculum that was standardized by matching the turbidity of the inoculum with 0.5 McFarland standard. A sterile swab stick was dipped into the inoculum and gently squeezed against the inside of the tube in order to remove excess fluid in the swab. The swab was used to inoculate sterile Mueller-Hinton (MH) agar plate, which was left to dry for about 5 mins. A sterile forcep was then used to place the antimicrobial-

impregnated disks on the surface of the agar plate, which was incubated at 37°C for 24 hours.

The diameter of zone of inhibition of the isolate to each antibiotic was measured with a calibrated ruler, and results interpreted as sensitive or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline (21). The following antibiotic discs (Oxoid, UK) were used; amoxicillin-clavulanate (30µg), gentamicin (10µg), ciprofloxacin (5 µg), nalidixic acid (30µg), ceftazidime (30µg), ceftriaxone (30 µg), nitrofurantoin (100µg), imipenem (10 µg), cefoxitin (30µg) and clindamycin (2µg).

Detection of ESBL in Gram-negative bacteria isolates:

Gram-negative bacterial isolates presumptively identified as ESBL-producing in the AST (ceftazidime zone diameter of inhibition < 22 mm or ceftriaxone zone < 25 mm) were confirmed as ESBL-producing by the double disk synergy test (21). The inoculum was prepared and agar plates inoculated as done for the AST. Ceftazidime (30µg) and ceftriaxone (30µg) discs were placed on either side of amoxicillin-clavulanic acid (20/10µg) 15 mm apart. ESBL-positive strains showed an expansion of the zone of inhibition of either cephalosporin toward the amoxicillin-clavulanate disk, giving a dumbbell shape (22).

Detection of methicillin resistance in *Staphylococcus aureus* isolates:

Mueller-Hinton agar plates were inoculated with *S. aureus* isolates as done for routine AST. Cefoxitin 30µg disks were applied and the plates were incubated at 37°C for full 24 hours. An isolate was considered to be an MRSA strain if the cefoxitin inhibition zone diameter was ≤ 21 mm (21).

Statistical analysis of data

Data were described in frequency and percentages. The Pearson Chi-square test was used to determine association of ASB with gender and age group of participants. P value < 0.05 was considered statistically significant at 95% confidence interval.

Results:

Three hundred HIV-positive individuals were enrolled into the study, with age range of 0-80 years, with female predominance (262, 87.3%). The CD4 counts of the participants ranged from 58 to 1082 cells/mm^3 (Table 1). Table 2 shows the results of culture of the urine samples, with samples from 67 (22.3%) individuals yielding significant bacteriuria, while 233 (77.7%) samples had either no growth or no significant growth, and 51 (17.0%) samples had mixed bacterial growth.

Staphylococcus aureus was the most frequently isolated bacteria (41.8%, 28/67), followed by *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa* and coagulase negative staphylococci CoNS), accounting for 25.4% (17/67), 17.9% (12/67), 7.5% (5/67), 4.5% (3/67) and 2.9% (2/67) respectively (Fig 1).

Table 3 shows the frequency distribution of ASB among the participants with respect to age group and gender. There was no significant association between ASB and age group of the participants (p=0.9632). Although, ASB prevalence was higher among the female participants (23.3%) compared to the male participants (15. 8%), the difference was not statistically significant (p=0.4077).

Table 4 shows the AST pattern of the isolates. *Staphylococcus aureus* was resistant to amoxicillin-clavulanate (64.3%), ceftriaxone (53.6%), ciprofloxacin (64.3%), and nalidixic acid (71.4%); *E. coli* was also resistant to amoxicillin-clavulanate (70.6%), ceftriaxone (53.6%), ciprofloxacin (52.9%), and nalidixic acid (64.7%); *K. pneumoniae* was moderately resistant to amoxicillin-clavulanate (50.0%), and resistant to ciprofloxacin (58.3%) and nalidixic acid (75.0%); *K. oxytoca* was resistant to amoxicillin-clavulanate (80.0%), gentamicin (60.0%), ciprofloxacin (60.0%) and nalidixic acid (80.0%); *P. aeruginosa* was resistant to gentamicin (100.0%), ciprofloxacin (66.7%), and nalidixic acid (100%); while coagulase negative staphylococci were resistant to nalidixic acid (100.0%) and moderately resistant to nitrofurantoin (50.0%) and ciprofloxacin (50.0%).

Discussion:

In this study, the prevalence of ASB was 22.3%. The observation of the relatively high prevalence of ASB in this study may require the need for laboratory investigation as a criterion for the commencement of treatment in HIV-infected patients. Our finding is in agreement with a study conducted in Osogbo, Nigeria which reported a prevalence of 23.5% (23), but higher than the rates of 11.9% reported in Gondar (24) and 10.7% in Jimma (4) Ethiopia, while the rate is lower than those reported from studies conducted in Ebonyi State, Nigeria with 93.8% (25), and in Tamil Nadu India, with 77.5% (26). The disparity in rates might be attributed to differences in sample size (small sample size might overestimate the proportion), geographical variation, and socioeconomic conditions.

The prevalence of ASB in our study participants appears to be much higher than those reported in other parts of the country and globally as most studies report prevalence of 3-15 % (27,28). Only a few studies such as those by Ojoo et al., (29) have reported relatively high prevalence as ours. Another study in this environment also reported a high prevalence of 18% among HIV-infected pregnant women (30). Compared with HIV-negative population within the same region, it appears that the prevalence of ASB is similar to what was obtained in our study population. A community-based study on HIV-negative individuals in the same region also reported a prevalence of 22.6%, which is comparable to our prevalence of 22.3% (31). Similar findings were also reported by Gugino et al., (32) and Widmer et al., (28) as they did not find any difference in the prevalence of ASB between their study population of HIV-positive women and HIV-negative controls.

Table 1: Age group and gender distribution of HIV-infected study participants attending selected HIV clinics in Ilorin, Nigeria

Gender	**Male (%)**	**Female (%)**	**Total (%)**
Age group (years)			
0-10	1 (0.3)	3 (1.0)	4 (1.3)
11-20	1 (0.3)	4 (1.3)	5 (1.7)
21-30	3 (1.0)	44 (14.7)	47 (15.7)
31-40	8 (2.7)	95 (31.7)	103 (34.3)
41-50	12 (4.0)	68 (22.7)	80 (26.7)
51-60	8 (2.7)	38 (12.7)	46 (15.3)
61-70	4 (1.3)	9 (3.0)	13 (4.3)
71-80	1 (0.3)	1 (0.3)	2 (0.7)
Total	**38 (12.7)**	**262 (87.3)**	**300 (100.0)**
CD4+ count (/mm^3)			
<200	8 (2.7)	86 (28.7)	94 (31.3)
200–500	18 (6.0)	120 (40.0)	138 (46.0)
>500	12 (4.0)	56 (18.7)	68 (22.7)
Total	**38 (12.7)**	**262 (87.3)**	**300 (100.0)**

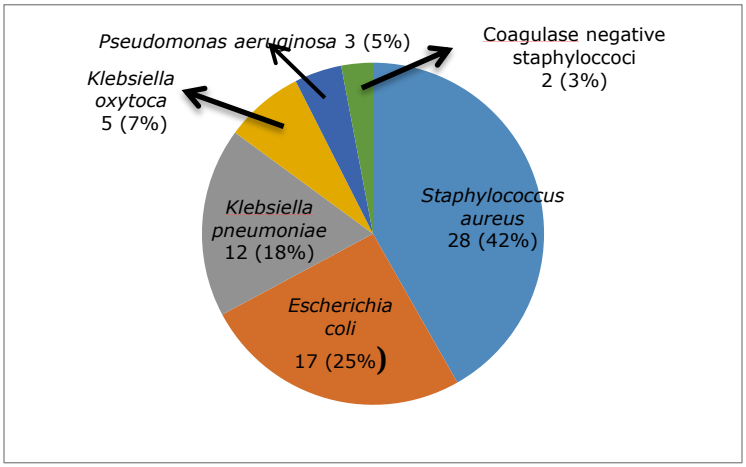

Fig 1: Frequency distribution of isolated bacteria from HIV-infected study participants attending selected HIV clinics Ilorin, Nigeria

Table 2: Frequency of bacteria growth from urine of HIV-infected study participants attending selected HIV clinics in Ilorin, Nigeria

Results of culture growth	Frequency of growth	Percentage
Significant growth	67	22.3
No significant growth	57	19.0
Mixed growth	51	17.0
No growth	125	41.7
Total	**300**	**100. 0**

Table 3: Association of ASB with gender and age group in HIV-infected participants attending selected HIV clinics in Ilorin, Nigeria

Demographic Characteristics	Significant bacteriuria			x^2	OR (95% CI)	*p* value
	Yes (%) [67 (22.3)]	No (%) [233 (77.7)]	Total (n=300)			
Age group (years)						
0-20	2 (22.2)	7 (77.8)	9	0.2828	NA	0.9632
21-40	32 (21.3)	118 (78.7)	150			
41-60	29 (23.0)	97 (77.9)	126			
61-80	4 (26.7)	11 (73.3)	15			
Gender						
Male	6 (15.8)	32 (84.2)	38	0.6856	0.6178 (0.2467-1.547)	0.4077
Female	61 (23.3)	201 (76.7)	262			

x^2 = Chi square; OR = Odd ratio; CI= Confidence interval; NA=Not applicable

The usual trend in ASB is for the prevalence to be higher in the female population (23), and our study also reported similar finding. We found the prevalence of asymptomatic bacteriuria to be higher in the females than males in keeping with reported trend, which is attributed to the proximity of the urethra to the anus and its short length (33). We, however could not establish statistically significant association (p= 0.4077) and therefore cannot conclude on such association in this group of HIV-infected individuals.

In the present study, the highest prevalence of ASB was recorded in the age group 61-80 years (26.7%) followed by age group 41-60 years (23.0%), age group 0-20 years (22.2%) and age group 21-40 years (21.3%). However, the association of ASB with age group was not statistically significant (p=0.9632). The ASB rate of 21.3% recorded in the age 21–40 years in our study is lower compared to the ASB rate of 53.9% reported in a slightly comparable age group 20–29 years in Irrua, Nigeria (34), but higher than 12.7% in the age group 18–26 years in Gondar Ethiopia (24).

Our study did not show significant difference in the ASB rate in the younger and older age groups in spite of the established fact that ASB rate tend to be higher in the younger age group in relation to the increased sexual activity of this age group, which is a recognized predisposition to bacteriuria and UTI (35). However, several studies have also reported that older age groups, especially the elderly, are also at risk of significant bacteriuria (31,35,36).

UTIs appear to be multifactorial in patients with HIV infections as $CD4^+$ level declines (37), and many research studies have reported this (38,39,40,41). This implies that the more immune compromised the patient is, the higher the risk of UTI and possibly to other opportunistic infections. The declining $CD4^+$ count makes it easier for bacterial pathogens to adhere to the urinary epithelium (27). Ezechi et al., (30) in their study on the risk factors for ASB in HIV-infected pregnant women also reported similar findings. Furthermore, they found high viral load, low haemoglobin, and previous UTI to be associated with ASB. However, we did not explore these parameters in our study.

Table 4: Antibiotic susceptibility of isolated bacteria from HIV-infected participants attending HIV clinics of selected Hospitals in Ilorin, Nigeria

Organisms	**Antibiotics Patterns (%)**								
	AMC	**CN**	**CIP**	**NA**	**CAZ**	**CRO**	**F**	**IPM**	**FOX**
Staphylococcus aureus (n=28)	S 10 (35.7)	15 (53.6)	10 (35.7)	8 (28.6)	NT	13 (46.4)	15 (53.6)	NT	20 (71.4)
	R 18 (64.3)	13 (46.4)	18 (64.3)	20 (71.4)	NT	15 (53.6)	13 (46.4)	NT	8 (28.6)
Escherichia coli (n=17)	S 5 (29.4)	13 (76.5)	8 (47.1)	6 (35.3)	14 (82.4)	14 (82.4)	12 (70.6)	NT	NT
	R 12 (70.6)	4 (23.5)	9 (52.9)	11 (64.7)	3 (17.6)	3 (17.6)	5 (29.4)	NT	NT
Klebsiella Pneumoniae (n=12)	S 7 (58.3)	6 (50.0)	5 (41.7)	3 (25.0)	7 (58.3)	7 (58.3)	10 (83.3)	NT	NT
	R 5 (41.7)	6 (50.0)	7 (58.3)	9 (75.0)	5 (41.7)	5 (41.7)	2 (16.7)	NT	NT
Klebsiella oxytoca (n=5)	S 1 (20.0)	2 (40.0)	2 (40.0)	1 (20.0)	3 (60.0)	3 (60.0)	5 (100)	NT	NT
	R 4 (80.0)	3 (60.0)	3 (60.0)	4 (80.0)	2 (40.0)	2 (40.0)	0	NT	NT
Pseudomonas aeruginosa (n=3)	S 2 (66.7)	1 (33.3)	0	0	2 (66.7)	3 (100)	3 (100)	3 (100)	NT
	R 1 (33.3)	2 (66.7)	3 (100)	3 (100)	1 (33.3)	0	0	0	NT
Coagulase negative staphylococci (n=2)	S 2 (100)	2 (100)	1 (50.0)	0	NT	2 (100)	1 (50.0)	NT	2 (100)
	R 0	0	1 (50.0)	2 (100)	NT	0	1 (50.0)	NT	0

AMC- augmentin, CN- gentamicin, CIP- ciprofloxacin, NA- nalidixic acid, CAZ- ceftazidime, CRO – ceftriaxone, F – nitrofurantoin, IPM – imipenem, FOX –cefoxitin, S – sensitive, R – resistant, NT – not tested

Staphylococcus aureus was the most predominant causative agent of ASB in the present study accounting for 41.8% of the uropathogens. A similar finding was reported from in Ebonyi State, Nigeria (25), and in Tamil Nadu, India (26), but contrasted the findings from Gondar (24) and Jimma (4) in Ethiopia, and in a tertiary care hospital in India (42), where *E. coli* was the mostly commonly isolated uropathogen, with frequency of 56.1%, 54.3% and 41.7% respectively. The variation in the type of bacteria isolates might be due to differences in sample size of studies, specimen collection techniques, sample processing methods, and personal and environmental hygiene factors (38).

Antimicrobial resistance is a major clinical challenge in treating infections caused by different bacterial pathogens and has increased over the years. In the present study, there was high resistance rates to some commonly used antibiotics, especially nalidixic acid, amoxicillin-clavulanate (augmentin), ciprofloxacin, and moderate resistance to gentamicin and ceftriaxone. This is to somewhat comparable with the study reports from Gondar (24) and Jimma (4) in Ethiopia. The resistance to the antibiotics may be due to the fact that some of these drugs are used for prophylaxis against opportunistic infections associated with HIV (43). The variations in the reports of resistance from the studies may be due to the differences in the distribution of resistant strains across the different settings.

Multidrug resistance has serious implications on the health outcome of HIV-infected patients (44,45). It is quite alarming to note that almost 40.8% of the isolates in this study were resistant to two or more antimicrobials. This is similar to a report of 46.0% by Dadi et al., (46) in Hiwot Fana Ethiopia, but higher compared to 28.0% reported in Mysore India by Murugesh et al., (45), and lower than the 95.0% reported in Gondar Ethiopia (24). The high resistance rate to the most commonly prescribed antibiotics in our study might be due to easy availability of these drugs in the community and their cheap costs, which make them subject to misuse (47). It could also be due to the use of antibiotics for other non-human purposes such as in livestock rearing and animal husbandry activities, which may be accelerating the growth of resistance (25,46).

The strength of our descriptive study is that it evaluated urine samples for pathogenic bacteria and highlighted the emergence of antimicrobial resistance that provides precise scientific data needed to develop strategy for the appropriate treatment, prevention, and control of UTI. However, we did not attempt to identify other causative agents (anaerobic bacteria, viruses, and fungi) that would have made a signi-

ficant contribution to the true prevalence of ASB in HIV-positive patients in our setting.

Conclusion:

In conclusion, the prevalence of ASB in HIV-infected persons in this study is relatively higher than some previous findings and common pathogens such as *S. aureus, E. coli*, and *K. pneumoniae* are the major agents of ASB. The isolation of high MDR bacteria highlights the growing challenge of UTIs, that is becoming increasingly difficult to treat with available antibiotics. Future studies need to focus on exploring a range of causative pathogens and the mechanisms of antimicrobial resistance.

Contributions of authors:

BMI designed the research and perform data analysis; SJP performed data analysis; BRA carried out the data collection and analysis; BSK reviewed the manuscript; AAB prepared the tables and figures. All authors reviewed the results and approved the final version of the manuscript.

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References:

1. Obeagu, E. I., Godfred, Y. S., Amekpor, F., Ofodile, A. C., Edoho, S. H., and Ahamefula, C. Prevention of new cases of human immunodeficiency virus: pragmatic approaches of saving life in developing countries. Madonna University Journal of Medicine and Health Sciences. 2022; 2 (3): 128-134.
2. Sewale, Y., Afenigus, A. D., Haile, D., and Shiferaw, W. S. Magnitude of hypertension and associated factors among human immunodeficiency virus positive adults receiving anti-retroviral therapy at Debre Markos Referral Hospital, Northwest, Ethiopia. HIV/AIDS-Research and Palliative Care. 2020: 629-637.
3. Abuto, W. Survival and predictors of mortality among HIV positive adult patients on highly active antiretroviral therapy in public hospitals of Kambata Tambaro Zone, Southern Ethiopia: a retrospective cohort study. HIV/AIDS Res Palliative Care. 2021: 271-281.
4. Debalke, S., Cheneke, W., Tassew, H., and Awol, M. Urinary tract infection among antiretroviral therapy users and non-users in Jimma University Specialized Hospital, Jimma, Ethiopia. Int J Microbiol. 2014; 6
5. Cunningham, A. L., Donaghy, H., Haman, A. N., Kim, M., and Turville, S. G. Manipulation of dendritic cell function by viruses. Curr Opin Microbiol.2010; 113: 524 - 529.
6. Kemajou, T. S., Ajugwo, A. O., Oshoma, C. E., and Enabulele, O. I. Antibiotic Resistance of Bacterial Isolates from HIV Positive Patients with Urinary Tract Infection (UTI) in Port Harcourt, Nigeria. J AIDS Clin Res. 2016; 7: 594.
7. Ani, O. C., Onwe, C. S., and Nnabugwu, C. C. Evaluation of female residents for urinary tract infections, Onicha Local Government Area, Ebonyi State, Nigeria. Nig J Parasitol. 2015; 36(1): 50-54.
8. Ramstedt, M., Ribeiro, I. A., Bujdakova, H., Mergulhão, F. J., Jordao, L., Thomsen, P., and Sjollema, J. Evaluating efficacy of antimicrobial and antifouling materials for urinary tract medical devices: Challenges and recommendations. Macromol Biosci. 2019; 19 (5): 1800384.
9. Katz, A. R. Urinary tract infections and acupuncture. Am Med J Publ Health. 2003; 9: 702-723.
10. Ovalle, A., and Levancini, M. Urinary tract infections in pregnancy. Curr Opin Urol. 2001; 11 (1): 55–59.
11. Foxman, B. Epidemiology of urinary tract infection: incidence, morbidity, and economic costs. Dis Mon. 2003; 49 (2): 53–70.
12. Iweriobor, B. C., Obi, C. L., and Akenyemi, O. Uropathogens isolated from HIV-infected patients from Limpopo Province, South Africa. Afr J Biotechnol. 2012; 11: 1059810604.
13. Samje, M., Yongwa, O., Enekegbe, A. M., and Njoya, S. Prevalence and antibiotic susceptibility pattern of bacteriuria among HIV-seropositive patients attending the Bamenda Regional Hospital, Cameroon. Afr Health Sci. 2020; 20(3): 1045-1052
14. Hidron, A. I., Kempker, R., Moanna, A., and Rimland, D. Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. Infect Drug Resist. 2010; 3: 73-86.
15. Gajdács, M. Increasing relevance of Gram-positive cocci in urinary tract infections: a 10-year analysis of their prevalence and resistance trends. Sci Rep. 2020; 10 (1): 1-11.
16. Timothy, O. O., Olusesan, F. J., Adesola, B. O., Temitayo, A. A., David, F. O., and Ige, O. O. Antibiotic resistance pattern of bacterial isolates from cases of urinary tract infections among hospitalized and out-patients at a tertiary health facility in South Western Nigeria. Ann Trop Med Publ Health.
17. Schlesselman, J. J. Sample size requirements in cohort and case-control studies of disease. Am J Epidemiol. 1974; 99 (6): 381–384. https://doi.org/10.1093/oxfordjournals.aje.a121625
18. Cheesbrough, M. Microbiology tests: District Laboratory practices in tropical Countries. Volume 2. Cambridge University Press, UK. 2006: 35-70.
19. Bale, M. I., Babatunde, S. K., and Awe, S. Prevalence of methicillin resistant *Staphylococcus aureus* bacteriuria among pregnant women attending secondary health hospitals in Ilorin, Nigeria. J Adv Microbiol. 2021; 21 (9): 50-57.
20. Fawole, M. O., and Oso, B. A. Characterization of Bacteria. In Laboratory Manual of Microbiology. Spectrum Books Limited, Nigeria. 2004; 22-33.
21. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 27th M100. Clinical and Laboratory Standards Institute; PA, USA. 2017
22. Bradford, P. A. Extended Spectrum β Lactamases in the 21st century: Characterization, Epidemiology and Detection of This Important Resistance Threat. Clin Microbiol Rev. 2001; 14: 933–951.
23. Olowe, O., Ojo-Johnson, B., Makanjuola, O., Olowe, R., and Mabayoje, V. Detection of bacteriuria among human immunodeficiency virus seropositive individuals in Osogbo, south-western Nigeria. Euro

J Microbiol Immunol. 2015; 5 (1): 126–130.
24. Alemu, A., Dagnew, M., Alem, M., and Gizachew, M. Uropathogenic bacterial isolates and their antimicrobial susceptibility patterns among HIV/AIDS patients attending Gondar University Specialized Hospital, Gondar, Northwest Ethiopia. J Microbiol Res Rev. 2013; 1(4): 42–51.
25. Ifeanyichukwu, I., Emmanuel, N., and Chika, E. Frequency and antibiogram of uropathogens isolated from urine samples of HIV infected patients on antiretroviral therapy. Am J Biosci. 2013; 1 (3): 50–53.
26. Xavier, T. F., Auxilia, A., and Kannan, M. Isolation and characterization of UTI pathogens from HIV positive patients of Karur District, Tamil Nadu, India. Int J Curr Microbiol Appl Sci. 2015; 558–563
27. De-Pinho, A. M., Lopes, G. S., Ramos-Filho, C. F., et al. M. Urinary tract infection in men with AIDS. Genitourin Med. 1994; 70: 30–34.
28. Widmer, T. A., Theron, G., and Grove, D. Prevalence and risks of asymptomatic bacteriuria among HIV-positive pregnant women. South Afr J Epidemiol. Infect. 2010; 25 (1): 28–32.
29. Ojoo, J., Paul, J., Batchelor, B., et al. Bacteriuria in a cohort of predominantly HIV-1 seropositive female commercial sex workers in Nairobi, Kenya. J Infect. 1996; 33: 33–37
30. Ezechi, O. C., Gab-Okafor, C. V., Oladele, D. O., et al. Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. J Matern-Fetal Neonat Med. 2013; 26 (4): 402–406
31. Olowe, O. A., Makanjuola, O. B., Olabiyi, K. O., Akinwusi, P. O., Alebiosu, C. O., and Isawumi, M. A. Asymptomatic bacteriuria among elderly and middle-aged rural community-dwellers in South-Western Nigeria. Infect. Drug Res. 2013; 6: 55–58
32. Gugino, L., Russo, T., Wactawski-Wende, J., Goodnough, S. L, Tristram, D. A., and Mylotte, J. Asymptomatic bacteriuria in human immunodeficiency (HIV)-infected women. Prim Care Update Ob Gyns. 1998; 5 (4): 146
33. Iduoriyekemwen, N. J., Sadoh, W. E, and Sadoh, A. E. Asymptomatic bacteriuria in HIV positive Nigerian children. J Med Biomed Res. 2012; 11 (1): 88–94.
34. Samuel, S., Salami, T., Adewuyi, G., Babatope, E., and Ekozien, M. Prevalence of urinary tract infections among a cohort of HIV positive patients accessing care in a rural health centre in Nigeria. J Microbiol Biotechnol Res Scholars. 2012; 2 (4): 507–510.
35. Hermann, V., Palma, P., Geo, M. S., and Lima, R. S. Urinary tract infections: pathogenesis and related conditions. Int Urogynecol J. 2002; 13: 210–213
36. Ariathianto, Y. Asymptomatic bacteriuria – prevalence in the elderly population. Aust Fam Physician 2011; 40, 805–809
37. Omoregie, R., and Eghafona, N. Urinary tract infection among asymptomatic HIV patients in Benin City, Nigeria. Br J Biomed Sci. 2009; 66 (4): 190–193.
38. Adebayo, A., and Salman, A. "Prevalence and predictors of asymptomatic bacteriuria in HIV positive pregnant women. J Med Med Sci Res. 2014; 3 (5): 48–54.
39. Akinbami, A., Bode-Shojobi, I., and Ajibola, S. Prevalence of asymptomatic bacteriuria in HIV infected patients in a Tertiary Hospital in Lagos, Nigeria. World J AIDS. 2013; 3 (2): 105–110.
40. Fenta, G., Legese, M., and Weldearegay, G. Bacteriuria and their antibiotic susceptibility patterns among people living with HIV attending Tikuran Bessa specialized and Zewditu memorial hospital ART clinics, Addis Ababa, Ethiopia. J Bacteriol Parasitol. 2016; 7 (5): 1–7.
41. Skrzat-Klapaczynska, A., Matlosz, B., and Bednarskal, A. Factors associated with urinary tract infections among HIV-1 infected patients. PLoS One. 2018; 13 (1): 2-18.
42. Banu, A., and Jyothi, R. Asymptomatic bacteriuria in HIV-positive individuals in a tertiary care hospital. J HIV Human Reprod. 2013; 1 (2): 54.
43. Lyamuya, E., Moyo, S., Komba, E., M., and Haule, M. Prevalence, antimicrobial resistance and associated risk factors for bacteriuria in diabetic women in Dar Es Salaam, Tanzania. Afr J Microbiol Res. 2011; 5 (6): 683–689.
44. Rashmi, K., Ravikumar, K., Nimitha, J., and Bhagyashree, H. Asymptomatic bacteriuria in HIV/AIDS patients: occurrence and risk associated with low CD4 counts. J Evol Med Dental Sci. 2013; 2 (19): 3358–3366.
45. Murugesh, K., Deepa, S., Ravindranath, C., and Venkatesha, D. Multidrug resistant uropathogens in HIV: are they a threat to community? Int J Sci Study. 2014; 2 (3): 38–42.
46. Dadi, M., Senthilkumar, B., and Berhanu, S. Prevalence, Antimicrobial Susceptibility Pattern of Bacterial Isolates, and Associated Factors of Urinary Tract Infections among HIV-Positive Patients at Hiwot Fana Specialized University Hospital, Eastern Ethiopia. Canadian J Infect Dis Med Microbiol. 2019; 19: 8.
47. Eyasu, M., Kassahun, K., Yilikal, A., Yewondwossen, T., and Alemayehu, K. Food Medicine and Healthcare Administration and Control Authority of Ethiopia, Addis Ababa, Ethiopia, 3rd edition. 2014

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Original Article **Open Access**

Automated blood culture systems for isolation of bacterial pathogens of bloodstream infection: The experience of Bobo-Dioulasso Teaching Hospital, Burkina Faso

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Abstract:

Background: Identification of the causative agent is an essential requirement for better treatment of bloodstream infection. The BacT/Alert 3D (BioMérieux, Marcy l'Étoile, France), is a blood culture system equipped with CO_2 sensors to monitor the growth of microorganisms in blood culture bottles designed to optimize bacterial growth. The aim of this study was to determine the performance of this equipment in detecting bacterial pathogens from patients with bloodstream infection in the context of low-and-middle-income countries (LMICs), with Bobo-Dioulasso Teaching Hospital as a case study.
Methodology: A cross-sectional study was conducted over a period of 5 months at the Sourô Sanou University Hospital, Bobo-Dioulasso, Burkina Faso, a low-income country. Blood samples from a total of 231 patients with clinical suspicion of bloodstream infections were collected and processed according to the manufacturer's instructions.
Results: Sixty-nine of the 231 blood culture samples of patients were positive, giving a bacteriological yield of 29.9%. *Escherichia coli, Salmonella* spp, and *Staphylococcus aureus* were the top three bacterial species isolated.
Conclusion: The implementation of the BacT/Alert 3D system has significantly enhanced the diagnosis of bacteraemia in the Bobo-Dioulasso Teaching Hospital. This enhancement is marked by time savings in patient care, reduced staff workload, and an increased bacteriological yield over time.

Keywords: Bloodstream infection, Automated blood culture, Low-and-middle-income-countries

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Systèmes automatisés d'hémoculture pour l'isolement des bactéries pathogènes des infections sanguines: l'expérience de Hôpital Universitaire de Bobo-Dioulasso, Burkina Faso

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Résumé:

Contexte: L'identification de l'agent causal est une démarche essentielle pour améliorer le traitement des sepsis.

Le système d'hémoculture BacT/Alert 3D (BioMérieux, Marcy l'Étoile, France) est équipé de capteurs de CO_2 pour surveiller la croissance des micro-organismes dans des flacons d'hémoculture conçus pour optimiser la croissance bactérienne. L'objectif de cette étude était de déterminer la performance de cet équipement dans la détection des pathogènes bactériens chez les patients présentant des signes d'un sepsis dans le contexte des pays à revenu faible et intermédiaire (PRFI), avec le Centre Hospitalier Universitaire (CHU) de Bobo-Dioulasso comme cas d'étude.

Méthodologie: Une étude transversale a été menée sur une période de 5 mois au CHU Sourô Sanou de Bobo-Dioulasso, Burkina Faso, un pays à faible revenu. Des échantillons de sang de 231 patients présentant une suspicion clinique de sepsis ont été prélevés et traités conformément aux instructions du fabricant.

Résultats: Soixante-neuf des 231 échantillons d'hémoculture étaient positifs, ce qui représente un rendement bactériologique de 29,9%. *Escherichia coli*, *Salmonella* spp et *Staphylococcus aureus* étaient les trois espèces bactériennes les plus fréquemment isolées.

Conclusion : L'acquisition du système BacT/Alert 3D a considérablement amélioré le diagnostic des bactériémies au CHU de Bobo-Dioulasso. Cette amélioration se reflète par un gain de temps dans les soins aux patients, une réduction de la charge de travail du personnel et une augmentation du rendement bactériologique au fil du temps.

Mots-clés: Sepsis, Hémoculture automatisée, Pays à revenu faible et intermédiaire.

Introduction:

In the realm of clinical infections, bacteraemia represent a grave concern (1). Their unchecked progression can escalate to sepsis, often marked by a graver prognosis. Mortality rates associated with sepsis span from 10 to 50%, contingent on the severity of the concomitant pathology (2). However, the clinical significance of bacteraemia is frequently underestimated in low-and-middle-income-countries (LMICs), especially in the sub-Saharan Africa. Within these regions, fevers are commonly misattributed to malaria or addressed through empirical antibiotic treatment due to limited access to microbiological diagnosis. This practice inadvertently fuels the surge of antimicrobial resistance (3–5).

A cornerstone for optimal management of bloodstream infections (BSIs) is the identification of the causative agent. Despite marked advancements in clinical microbiology diagnostics, blood culture remains the 'gold standard' method for diagnosis of BSIs (6). In response to the inherent limitations of manual and semi-automated blood culture techniques, automated diagnostic systems that are reliant on continuous bacterial growth monitoring have emerged. Within this array of blood culture systems, the BacT/Alert 3D system (BTA3D, BioMérieux, Marcy l'Étoile, France) stands out, employing CO_2 sensors for growth surveillance. This system employs culture media designed to address inherent blood culture challenges (7).

Ordinarily, blood is sterile and contains antimicrobial elements that suppress bacterial proliferation during infections. Augmenting BC sensitivity thus mandates substantial blood volumes drawn from 2-4 pairs of bottles (6). Regrettably, in Burkina Faso and similar LMICs, adhering to these recommendations is problematic due to patients' limited financial means and the absence of universal health insurance, leading to cost burdens. Consequently, a single blood sample for two blood culture bottles is a common practice (8).

Considering these constraints, we seek to uncover the potential contribution of this automated blood culture system in practical usage. Here, we present our experience with the BTA3D, acquired amid resource constraints in the preceding one year. This study aims to highlight the performance of this equipment in the identification of bacterial pathogens from clinical cases of BSIs within the context of the Bobo-Dioulasso Teaching Hospital, Burkina Faso.

Materials and method:

Study setting:

Burkina Faso, situated in West Africa, represents a low-income country with an estimated population of 21,840,865 in the year 2022 (9). A substantial segment of its population (45.3%) resides below the poverty threshold of annual $ 115 per adult. The country's health infrastructure is underdeveloped, particularly in terms of diagnostic capabilities within laboratories. These diagnostic deficiencies hinder the effective management of prevalent infectious diseases. Compounded by the challenges of poverty and malnutrition, the prognosis of such diseases is further complicated in this resource-constrained setting (10,11).

Study design:

This was a hospital-based cross-sectional study conducted from October 1, 2015, to February 29, 2016, on patients with clinical features of bloodstream infection from whom blood culture samples were aseptically collected and submitted for routine analysis at the Department of Bacteriology laboratory of the Bobo-Dioulasso Teaching Hospital, Burkina Faso.

Blood sampling, inoculation of culture bottle, and incubation in BacT/Alert 3D:

Blood samples were collected from hospitalized patients with extreme body temper-

atures (>38°C or ≤ 36°C) as indicative of potential BSIs. A tourniquet was applied to the arm, followed by vein palpation and antiseptic application at the puncture site.

A sterile approach was maintained after antisepsis. Blood was drawn using a needle and syringe (or butterfly needle) and dispensed into specific blood culture bottles. Approximately 20 ml of blood was collected from adults, and dispensed into one aerobic (FAN adult, BioMérieux) and one anaerobic (FAN adult anaerobic, BioMérieux) culture bottles. About 5 ml was collected from paediatric patients into a paediatric blood culture bottle (FAN paediatric, BioMérieux). Established protocols were adhered to (6,7,12). Seeded blood culture bottles were placed in the BacT/Alert 3D system (BTA3D) for incubation at 37°C for up to 7 days, optimizing microbial growth detection.

Subculture of positive blood culture bottles:

Upon detection of positive blood cultures by the BacT/Alert 3D system, Gram stain microscopic examination was performed which guided subculture on suitable agar media. Subculture media were incubated at 37°C aerobically for 18-24 hours.

Microbial identification and antimicrobial susceptibility testing:

Microbial identification from positive cultures was achieved through culture morphology and biochemical identification tests (13). For Enterobacterales and non-fermenting Gram-negative bacilli identification, API 20 E and API 20 NE strips (BioMérieux) were respectively utilized. For Gram-positive cocci, selective media and conventional biochemical tests were employed for identification. Culture results were evaluated by a senior biologist to distinguish between causative pathogens and contaminants.

Antimicrobial susceptibility testing (AST) was performed on pathogenic isolates by the modified Kirby-Bauer disc diffusion method in line with the 2015 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (14). Internal quality control measures were employed to assess all stages of the blood culture test, encompassing incubation, solid media preparation, Gram staining, biochemical identification and AST. This process utilized reference strains, adhering to the EUCAST guideline (14).

Data management and ethical considerations:

Data collection was conducted from the blood culture registry and recorded in Microsoft Excel software version 2015. Patient anonymity and confidentiality were rigorously maintained. The study was smoothly incorporated into the standard care delivery framework and did not interfere with patient treatment. Given its alignment with routine healthcare procedures and the absence of any negative impact on patient management, no evaluation by the Research Ethics Committee was required in accordance with national regulations.

Results:

Patients' characteristics

A total of 231 requests for blood culture were received during the study period from 231 patients, with a mean age of 41±12 years and a gender ratio of 1.17. Most blood culture requests were from the Department of Paediatric Medicine (61%), followed by Department of Infectious Diseases (11%) and the Intensive Care Units (7%).

Bacteriological yield:

Sixty-nine blood culture bottles were positive, giving a bacteriological yield of 29.9%. This yield remained approximately the same regardless of the gender or age group of the patients. One patient had positive growth in both anaerobic and aerobic bottles but only the aerobic bottle was evaluated by sub-culture. All subcultures were positive, yielding 69 bacterial isolates belonging to 10 different species as shown in Table 1**.**

The morphological groups of bacteria isolated were almost equally represented with 50.8% being Gram-negative bacilli and 49.2% Gram-positive cocci. *Escherichia coli, Salmonella* spp., and *Staphylococcus aureus* were the three most frequently isolated species. *Staphylococcus epidermidis* was isolated in 14.4% of positive subcultures.

Results of antibiotic susceptibility testing:

The antibiotic susceptibility of *E. coli, Salmonella* spp*.,* and *S. aureus* strains is as shown in Table 2. *Salmonella* spp*.*, showed overall good sensitivity to ciprofloxacin (90. 9%) and gentamicin (100.0%). They were resistant to penicillin and cephalosporins in 80.9% and 36.4% of cases respectively. *S. aureus* was resistant to cefoxitin in 5.8% of cases, which was associated with resistance to fluoroquinolones and aminoglycosides in 58.9% and 60.6% of cases respectively. *E. coli* isolates were resistant to at least one penicillin, one cephalosporin, and imipenem in 93.6%, 80%, and 60.0% of cases respectively.

Table 1: Distribution of isolated bacterial pathogens from blood cultures of patients with bloodstream infection at Sourô Sanou Teaching Hospital, Bobo-Dioulasso, Burkina Faso

Bacterial species		**Number of isolates**	**Proportion (%)**
Gram positive cocci (n=34)	*Staphylococcus aureus*	17	24.6
	Staphylococcus epidermidis	10	14.4
	Enterococcus spp.	07	10.1
Enterobacterales (n=31)	*Klebsiella pneumoniae*	01	1.5
	Citrobacter freundii	01	1.5
	Escherichia coli	15	21.7
	Enterobacter spp.	03	4.4
	Salmonella spp.	11	15.9
Non fermentative (n=4)	*Acinetobacter* spp.	02	2.8
	Pseudomonas spp.	02	2.8
Total		**69**	**100.0**

[1] n: number

Table 2: Antibiotic susceptibility of the three main bacteria pathogens isolated from blood cultures of patients with bloodstream infection at Sourô Sanou Teaching Hospital, Bobo-Dioulasso, Burkina Faso

Bacterial isolates	**Percentage of isolates sensitive to antibiotics**										
	AMC	AX	CAZ	GM	CP	CN	IM	L	ER	FOX	PG
E. coli (n=15)	20	6.6	20	26.6	66.6	46.6	40	-	-	-	-
Salmonella spp (n=11)	36.3	9.1	72.7	63.6	90.9	100	45.4	-	-	-	-
S. aureus (n=17)	-	-	-	-	41.1	29.4	-	52.9	17.6	5.8	17.6

AX: amoxicillin; AMC: amoxicillin + clavulanic acid; PG: penicillin G; CZ: ceftriaxone; CAZ: Ceftazidime; GM: gentamicin; CP: ciprofloxacin; IM: imipenem; FOX: Cefoxitin; ER: erythromycin; L: Lincomycin; -: Not performed

Discussion:

This study aimed to comprehensively assess the utility of the automated BacT/Alert 3D system (BTA3D) in comparison to manual detection systems for BSIs within the specific setting of the Bobo-Dioulasso Teaching Hospital, one year following equipment acquisition. The investigation predominantly centered on elucidating potential improvements in bacteriological yield, elucidating the advantages of the BTA3D, addressing contamination reduction, streamlining patient care, reducing staff workload, and mitigating healthcare costs in the context of limited resources.

Enhanced bacteriological performance:

Within the period of study, a total of 231 blood cultures were processed, with 69 of them testing positive, giving a bacteriological yield of 29.9%. This reported yield aligns closely with findings from comparable studies utilizing automated systems (15,16). For instance, Okomo et al., (17) conducted research in Gambia in 2011, reporting a bacteriological yield of 27.1%. This yield outperformed analogous studies by Ki-Zerbo et al., (18), Ouédraogo et al., (19) and Bahwere et al., (20) which reported yields of 19.8%, 18.3% and 15.9% respectively. Noteworthy is that these studies were conducted within similar contextual constraints and employed manual detection methods.

The substantial deviation of approximately 10 percentage points underscores the pivotal contribution of automated systems in elevating bacteriological performance. The pronounced efficiency witnessed with the BTA3D system can be attributed, in part, to the composition of the blood culture media. In contrast to the studies cited above, the BTA3D blood bottles encompass a unique blend of saponin, resin, absorbent polymeric bile, sodium polyanetholsulfonate, and complex amino acid substrates (7,12). Saponin functions as a lytic agent, facilitating the release of intracellular microorganisms without impeding their growth. The presence of resin neutralizes antimi-

crobial activity in blood and augments surface area, thereby promoting microbial growth in biofilms. These attributes collectively augment the positivity rate of the automated method (2,12). Conversely, the hemoline media employed in studies by Ouedraogo et al., (18) in Burkina Faso in 2012 and Bahwere et al., (20) in Congo in 2001 lack some of the key components present in the BTA3D blood culture bottles.

Superiority of the BTA3D:

Our series exhibited a marginal preponderance of Gram-negative bacilli (GNB) at 50.8% (comprising Enterobacterales and non-fermenting organisms) juxtaposed with 49.2% Gram-positive cocci (GPC). These findings parallel those reported by Elouennass et al., (21) in Morocco in 2008 with 49.3% GNB and 46.8% GPC. However, Karlowsky et al., (22) in the US in 2004 reported an opposing trend with GPC dominance (78.1%). The divergence in results can be attributed to the distinct bacterial ecology and population characteristics.

The prevalence of children and infants in our study population likely contributes to a higher propensity for GNB-associated bacteraemia such as salmonellosis, in line with findings from other developing countries (21,23). Comparing the BTA3D to other automated blood culture systems highlights its discernible efficacy in detecting Enterobacterales. This proficiency is particularly advantageous in regions where limited hygiene and water access culminate in gastroenteritis and subsequent bacteraemia (5,17).

Contamination mitigation:

The issue of contamination in blood culture is a critical concern in the diagnosis of bloodstream infections. Coagulase-negative staphylococci, while capable of causing BSIs, are often indicative of contamination, accounting for approximately 70% of cases (27). In our study, we observed a coagulase-negative staphylococci rate of 14.4%, which could be closely aligned with the actual contamination rate within our study. However, it significantly exceeds the expected 2-5% rate reported in the literature for developed countries (28). Conversely, when compared to 19.3% contamination rate reported by El Kettani et al., (29) in 2017, this finding exhibits a slight discrepancy.

This minor variation may be attributable to variances in sampling techniques and the inherent higher risk of contamination associated with manual detection methods. Daily manual assessment of blood cultures involves frequent handling of culture bottles, increasing susceptibility to contaminants from commensal and environmental human flora, particularly enteric bacteria (28).

Clinical efficiency and staff workload:

The study yielded zero false positives when compared to subculture results. This outcome is particularly promising, as it enables prompt validation of clinical suspicions regarding the infectious origin of fever without awaiting complete identification and antimicrobial susceptibility testing (30). Such an approach expedites treatment modifications while awaiting comprehensive results. Additionally, the automated detection capability curtails laboratory personnel workload.

Manual methods necessitate daily visual scrutiny of blood culture bottles to identify those conducive to bacterial growth, engendering a higher likelihood of errors, especially among inexperienced staff. The BTA3D, with its CO_2 sensor-based detection mechanism, minimizes error risk and work burden, thus optimizing resource allocation within LMICs (24).

The reduction of costs of care for sepsis in the context of poverty:

The augmented sensitivity and swifter etiological diagnosis of fevers facilitated by the BTA3D system can potentially mitigate inpatient treatment costs. Given the absence of widespread blood culture availability in Burkina Faso, febrile syndromes are often treated empirically, leading to inappropriate use of expensive broad-spectrum antibiotics (5), contributing to both exacerbation of antimicrobial resistance and impoverishment of the family due to the lack of universal health insurance (11).

By enhancing the likelihood of identifying the causative agent in a timelier manner, the BTA3D can contribute to shorter hospital stays and more judicious resource utilization, thereby alleviating the financial burden associated with ineffective treatments.

Study limitations and perspectives:

Subcultures of bottles reported negative by BTA3D could not be performed due to lack of resources. Such cultures would help determine the false negative rate. Therefore, a study comparing the BTA3D to another device in the same line would better highlight the strengths and weaknesses of this equipment. In addition to the large volume of blood required, this experiment requires financial resources that are lacking in the Burkina Faso context.

Conclusion:

Blood culture continues to hold its significance as a crucial diagnostic tool for establishing the microbiological basis of bacteraemia. However, manual detection methods in LMICs exhibit suboptimal performance and are burdened with time-intensive processes.

The integration of the BTA3D automated detection system has emerged as a transformative step in enhancing the diagnosis of bacteraemia, as evidenced by the observed yield of 29.9% at the Bobo-Dioulasso Teaching Hospital.

This study underscores the substantial improvements brought about by the BTA3D, addressing both efficiency and accuracy concerns associated with conventional manual methods. Nonetheless, a comprehensive evaluation that directly compares the BTA3D with another device within the same domain would provide a more nuanced understanding of its capabilities and limitations. Such an assessment holds the potential to contribute to the advancement of bacteraemia diagnosis and management strategies, offering valuable insights for clinical practice in LMICs and beyond.

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Contributions of authors:

AN was involved in the data analysis, and writing of the manuscript; MK was involved in data compilation; ODK, BS, and CY were involved in the writing of the manuscript; ASO was involved in the supervision of the project and correction of the manuscript; IT, JZ, and AO were involved. All authors approved the final manuscript submitted.

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Conflicts of interest:

Authors declare no conflict of interest.

References:

1. Keita-Perse, O., and Gaynes, R. P. Severity of illness scoring systems to adjust nosocomial infection rates: A review and commentary. Am J Infect Contr. 1996; 24 (6): 429–434. https://doi.org/10.1016/S0196-6553(96)90036-X
2. Guimaraes, A. O., Cao, Y., Hong, K., et al. A Prognostic Model of Persistent Bacteremia and Mortality in Complicated *Staphylococcus aureus* Bloodstream Infection. Clin Infect Dis. 2019; 68 (9): 1502–1511. https://doi.org/10.1093/cid/ciy739
3. Obaro, S., Lawson, L., Essen, U., et al. Community Acquired Bacteremia in Young Children from Central Nigeria- A Pilot Study. BMC Infect Dis. 2011; 11 (1). https://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-11-137
4. Petti, C. A., Polage, C. R., Quinn, T. C., Ronald, A. R., and Sande, M. A. Laboratory Medicine in Africa: A Barrier to Effective Health Care. Clin Infect Dis. 2006; 42(3):377–382.https://doi.org/10.1086/499363
5. Kiemde, F., Tahita, M. C., Lompo, P., et al. Treatable causes of fever among children under five years in a seasonal malaria transmission area in Burkina Faso. Infect Dis Poverty. 2018; 7 (1): https://doi.org/10.1186/s40249-018-0442-3open_in_new
6. Lee, A., Mirrett, S., Reller, L. B., and Weinstein, M. P. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? J Clin Microbiol. 2007; 45 (11): 3546–3548. https://doi.org/10.1128/jcm.01555-07
7. Kirn, T. J., Mirrett, S., Reller, L. B., and Weinstein, M. P. Controlled Clinical Comparison of BacT/Alert FA Plus and FN Plus Blood Culture Media with BacT/Alert FA and FN Blood Culture Media. J Clin Microbiol. 2014; 52 (3):839–843. https://doi.org/10.1128/jcm.03063-13
8. Bonko, M., dit, A., Tahita, M. C., et al. Antibiotic susceptibility profile of bacterial isolates from febrile children under 5 years of age in Nanoro, Burkina Faso. Trop Med Int Health. 2021; 26 (10): 1220–1230. https://doi.org/10.1111/tmi.13644
9. Cinquième Recensement Général de la Population et de l'Habitation du Burkina Faso Ouagadougou, Burkina Faso: Comité National du Recensement, Institut National de la Statistique et de la Démographie; 2022: 136. http://www.insd.bf/contenu/documents_rgph5/Rapport%20resultats%20definitifs%20RGPH%202019.pdf (Accessed Sept 2, 2022)
10. Da, L., Somé, D., Yehouenou, C., et al. État des lieux de la résistance aux antibiotiques en Afrique subsaharienne. Médecine Mal Infect Form. 2023; 2 (1):3–12. https://doi.org/10.1016/j.mmifmc.2023.01.003
11. Ouedraogo, A. S., Jean Pierre, H., Bañuls, A. L., Ouédraogo, R., and Godreuil, S. Emergence and spread of antibiotic resistance in West Africa: contributing factors and threat assessment. Médecine Santé Trop. 2017; 27 (2): 147–154. https://doi.org/10.1684/mst.2017.0678
12. Flayhart, D., Borek, A. P., Wakefield, T., Dick, J., and Carroll, K. C. Comparison of BACTEC PLUS Blood Culture Media to BacT/Alert FA Blood Culture Media for Detection of Bacterial Pathogens in Samples Containing Therapeutic Levels of Antibiotics. J Clin Microbiol. 2007; 45 (3): 816–821. https://doi.org/10.1128/jcm.02064-06
13. Giménez, M., Prat, C., Vallés, X., Matas, L., Arnal, J., and Ausina, V. Evaluation of the VITAL (bioMérieux) automated blood culture system using blind subculture. Clin Microbiol Infect. 2002; 8 (4): 222-228 https://doi.org/10.1046/j.1469-0691.2002.00417.x
14. CASFM. Librairie Lavoisier. Comité de l'antibiogramme de la Société Française de Microbiologie (CASFM). 2015. https://www.lavoisier.fr/livre/notice.asp?ouvrage=3320903. (Accessed Sept 14 2022).
15. Jacobs, M. R., Mazzulli, T., Hazen, K. C., et al. Multicenter Clinical Evaluation of BacT/Alert Virtuo Blood Culture System. J Clin Microbiol. 2017; 55 (8): 2413–2421.https://doi.org/10.1128/jcm.00307-17
16. Mallat, H., Grohs, P., Levy, A., and Mainardi, J. L. Étude rétrospective des bactériémies diagnostiquées aux urgences : fréquence, sensibilité des micro-organismes et intérêt dans la prise en charge thérapeutique. Médecine Mal Infect. 2004; 34(7): 310–315.https://doi.org/10.1016/j.medmal.2004.06.001
17. Okomo, U. A., Garba, D., Fombah, A. E., et al. Bacterial Isolates and Antibiotic Sensitivity among Gambian Children with Severe Acute Malnutrition. Int J Pediatr. 2011; 2011: 1–8. https://doi.org/10.1155/2011/825123
18. Bahwere, P., Levy, J., Hennart, P., et al. Community-acquired bacteremia among hospitalized children in rural Central Africa. Int J Infect Dis. 2001; 5 (4): 180–188. https://doi.org/10.1016/S1201-9712(01)90067-0
19. Ki-Zerbo, G. A., Thioub, B., Diop, B. M., Badiane, S., Coll-Seck, A. M., and Samb, A. Étude des hémocultures positives au chu de fann Dakar: Bilan de trois années du laboratoire de bactériologie. Médecine Afr Noire. 1996; 8.

20. Ouédraogo, A. S., Dakouré-Kissou, A., Poda, G. E. A., Koueta, F., Yé-Ouattara, D., and Ouédraogo-Traoré, R. Aspects épidémiologiques, microbiologiques et évolutifs des bactériémies de l'enfant au Centre hospitalier universitaire pédiatrique Charles de Gaulle (Burkina Faso). Cah Détudes Rech Francoph Santé. 2011; 21 (4): 221–225. doi: 10.1684/san.2011.0273
21. Elouennass, M., Sahnoun, I., Zrara, A., Bajjou, T., and Elhamzaoui, S. Épidémiologie et profil de sensibilité des isolats d'hémoculture dans un service de réanimation (2002–2005). Médecine Mal Infect. 2008; 38 (1): 18–24. https://doi.org/10.1016/j.medmal.2007.10.006
22. Karlowsky, J. A., Jones, M. E., Draghi, D. C., Thornsberry, C., Sahm, D. F., and Volturo, G. A. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbiol Antimicrob. 2004; 8. https://ann-clinmicrob.biomedcentral.com/articles/10.1186/1476-0711-3-7
23. Gbadoé, A. D., Lawson-Evi, K., Dagnra, A. Y., et al. Les salmonelloses chez l'enfant au CHU Tokoin de Lomé (Togo). Médecine Mal Infect. 2008; 38(1): 8-11 https://doi.org/10.1016/j.medmal.2007.08.002
24. Totty, H., Ullery, M., Spontak, J., et al. A controlled comparison of the BacT/ALERT® 3D and VIRTUO™ microbial detection systems. Eur J Clin Microbiol Infect Dis. 2017; 36 (10): 1795–1800. https://doi.org/10.1007/s10096-017-2994-8
25. Mirrett, S., Reller, L. B., Petti, C. A., et al. Controlled Clinical Comparison of BacT/ALERT Standard Aerobic Medium with BACTEC Standard Aerobic Medium for Culturing Blood. J Clin Microbiol. 2003; 41 (6): 2391–2394. https://doi.org/10.1128/jcm.41.6.2391-2394.2003
26. Hellinger, W. C., Cawley, J. J., Alvarez, S., et al. Clinical Comparison of the Isolator and BacT/Alert Aerobic Blood Culture Systems. J Clin Microbiol. 1995; 33 (7): 1787-1790. https://doi.org/10.1128/jcm.33.7.1787-1790.1995
27. Sidhu SK., Malhotra S., Devi P., Tuli AK. Significance of coagulase negative Staphylococcus from blood cultures: persisting problems and partial progress in resource constrained settings. Iran J Microbiol. 2016; 8 (6): 366–371.
28. Hall, K. K., and Lyman, J. A. Updated Review of Blood Culture Contamination. Clin Microbiol Rev. 2006; 19 (4): 788–802. https://doi.org/10.1128/cmr.00062-05
29. El Kettani, A., Zerouali, K., Diawara, I., et al. Les bactériémies associées aux soins en réanimation au Centre hospitalier universitaire Ibn Rochd, Casablanca, Maroc. Santé Publique. 2017; 29 (2): 209. https://doi.org/10.3917/spub.172.0209
30. Szabados, F., Michels, M., Kaase, M., and Gatermann, S. The sensitivity of direct identification from positive BacT/ALERT™ (bioMérieux) blood culture bottles by matrix-assisted laser desorption ionization time-of-flight mass spectrometry is low. Clin Microbiol Infect. 2011; 17 (2): 192–195. https://doi.org/10.1111/j.1469-0691.2010.03229.x

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Original Article **Open Access**

Bioactive components of *Syzygium aromaticum* bud and their effects on selected pathogenic bacteria

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Abstract:

Background: The decline in the effectiveness of common antibiotics is due to microbial resistance and has sparked research interest in discovering new antimicrobial agents from plants. The objective of this study was to evaluate the effectiveness of clove extracts on selected pathogenic bacteria and identify the active antibacterial components.
Methodology: The active components of *Syzygium aromaticum* (clove) buds were extracted using methanol and ethyl acetate and identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Antibacterial screenings against selected pathogenic bacteria were conducted using the agar-well diffusion method.
Results: The extracts showed activities against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at 100 mg/ml, so that the diameters of the methanol extracts were 4.00-25.00 mm, 3.00-26.00 mm and 0.00 -16.00 mm, while the ethyl acetate extracts were 3.00-24.00 mm, 4.00-22.00 mm and 0.00-14.00 mm respectively. The ethyl acetate extract showed higher MIC and MBC at 3.125 and 6.5 mg/mL and 12.5 mg/mL, indicating a more lethal effect than the methanol extract. Nineteen bioactive compounds were identified the extracts.
Conclusion: The study justifies the use of the clove plant by traditional herbalists to treat bacterial infections due to the presence and synergy of the plant's various bioactive components.

Keywords: *Syzygium aromaticum*, Clove extract, Antibacterial activity, bioactive compounds

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Composants bioactifs du bourgeon de *Syzygium aromaticum* et leurs effets sur certaines bactéries pathogènes

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Résumé:

Contexte: Le déclin de l'efficacité des antibiotiques courants est dû à la résistance microbienne et a suscité l'intérêt de la recherche pour la découverte de nouveaux agents antimicrobiens à partir de plantes. L'objectif de cette étude était d'évaluer l'efficacité des extraits de clou de girofle sur des bactéries pathogènes sélectionnées et d'identifier les composants antibactériens actifs.

Méthodologie: Les composants actifs des bourgeons de Syzygium aromaticum (clou de girofle) ont été extraits à l'aide de méthanol et d'acétate d'éthyle et identifiés par analyse par chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). Des criblages antibactériens contre des bactéries pathogènes sélectionnées ont été effectués en utilisant la méthode de diffusion en puits d'agar.
Résultats: Les extraits ont montré des activités contre *Staphylococcus aureus, Escherichia coli* et *Pseudomonas aeruginosa* à 100 mg/ml, de sorte que les diamètres des extraits au méthanol étaient de 4,00-25,00 mm, 3,00-26,00 mm et 0,00-16,00 mm, tandis que les extraits à l'acétate d'éthyle étaient de 3,00-24,00 mm, 4,00- 22,00 mm et 0,00-14,00 mm respectivement. L'extrait à l'acétate d'éthyle a montré des CMI et MBC plus élevés à 3,125 et 6,5 mg/mL et 12,5 mg/mL, indiquant un effet plus létal que l'extrait au méthanol. Dix-neuf composés bioactifs ont été identifiés dans les extraits.
Conclusion: L'étude justifie l'utilisation du giroflier par les herboristes traditionnels pour traiter les infections bactériennes du fait de la présence et de la synergie des différents composants bioactifs de la plante.

Mots-clés: *Syzygium aromaticum*, extrait de clou de girofle, activité antibactérienne, composés bioactifs

Introduction:

Cloves (*Syzygium aromatum* L.) are a tree in the family Myrtaceae, which contains 1200 to 1800 species (1). The plant is grown in Nigeria and other parts of the world and its flower buds produce essential oil used as a spice in cooking and in traditional herbal medicine (2,3). The plant has been found to contain many phyto -chemicals, monoterpenes and eugenol, which have antimicrobial effects that can be studied to combat diseases caused by pathogenic micro-organisms.

Antibiotics have evolved from a purely microbial product to synthetic substances that inhibit pathogenic microorganisms (4). Antibiotics have helped to treat and control the spread of infectious diseases and curbed infections in surgical and pediatric patients, thereby preventing high mortality rates for decades (5). Today, the benefits of antibiotics extend to various areas of human activity, including medical practice and agriculture (4). However, selective pressure from drug abuse has created resistance strains that can evade antibiotic action (6).

The spread of resistant bacteria poses a threat to medicine and is one of the main reasons for the worldwide decline in the effectiveness of antibiotics. In addition, multidrug resistance has increased mortality rates and led to the spread of diseases, particularly in African populations who were unaware of their effects (7). This requires research to focus on the discovery of herbal therapeutics. Therefore, this study was conducted to evaluate the effectiveness of clove extracts on selected pathogenic bacteria and identify the active components.

Material and method:

Study area:

This research was conducted at the Microbiology Laboratory of IBB University in Lapai, Niger State, Nigeria. The clove buds were collected from a community within longitude (9º03 N) and latitude (6º34 E) with an estimated population of 120,000 people, most of whom are farmers and heavy users of herbal medicines (8).

Sample collection and preparation:

Fresh cloves (*Syzygium aromaticum* L.) were purchased in bags and authenticated in the Department of Biological Sciences of the University. They were then washed in distilled water and dried at ambient temperature. The dry flower buds were ground, weighed and stored in a sterile container for experiments. The laboratory repository of the Department of Biochemistry, IBB University in Lapai provided the standard grade methanol and ethyl acetate utilized for the extraction of bioactive components from cloves.

Pure isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from stock cultures at the Microbiology Laboratory of IBB University Lapai Niger State. Isolates were sub-cultured onto nutrient agar plates to assess viability and obtain fresh cultures for the research.

Extraction of cloves flower buds:

The cold maceration method was used to extract the sample ingredients (9). Three hundred grams of the sample powder was transferred to 1000 ml of methanol in a 2-liter conical flask. The same procedure was repeated for ethyl acetate and each flask was sealed with cotton wool, wrapped in aluminum foil and allowed to stand for 48 hours. The flasks were shaken intermittently to achieve maceration and the filtrate was separated through Whatman No. 1 filter paper. The solvents were evaporated on a steam bath leaving only the extract in the container. Then, the yield of the extracts was calculated using the formula; % yield = weight of the plant extract/weight of dry sample (x 100).

Antibacterial activity of plant extracts:

The antibacterial activity of the extracts was determined by the agar diffusion method from zones of inhibition formed around the agar wells due to diffusion against the test organisms (10). Fresh nutrient agar plates were aseptically inoculated with 0.1 ml bacterial culture using the

spread-plate technique before making wells in the agar with a sterile 6 mm cork-borer. One gram of extract was introduced into six separate beakers with different volumes of dimethyl sulfoxide (DMSO) to give 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml of extracts. A positive control with the same concentrations of ciprofloxacin and a negative control with sterile water were setup.

The agar wells were filled with 1 ml of the different concentrations of the extracts and ciprofloxacin. The plates were incubated at 37°C for 24 hours and the diameters of the zone of inhibition around the agar wells were measured with a meter ruler. The experiment was performed in triplicate.

Minimum inhibitory concentration (MIC) by tube dilution method:

The MIC of the extracts was determined according to the tube dilution method of Adegbenu et al., (11). The extracts were serially diluted with DMSO in separate beakers to obtain different concentrations of the extracts (100, 50, 25, 12.5, 6.25 and 3.125 mg/ml). Five milliliters of each concentration were then dispensed into separate test tubes, followed by the addition of 5 ml of Muller-Hilton broth and shaken to mix. The tubes were inoculated with 0.2 ml bacterial solution and examined for the lower concentration of the extracts that showed no turbidity after an incubation period of 24 hours at 37°C

Minimum bactericidal concentration (MBC):

In the MIC study above, test tubes showing no growth were aseptically pour-plated onto fresh nutrient agar. After incubation at the same temperature for 24 hours, the plates were monitored and checked for observable colonies of the test organisms and the lowest concentration that showed no growth was designated MBC.

Identification of compounds by GC-MS analysis:

The oil compounds were identified by GC-MS analysis as described by Ibrahim et al., (12). One ml of the diluted oil was analyzed by GC-MS equipped with Agilent Split/Splitless and a BP5 (30m) equipped with a (0.25m 0.25 m) capillary column (30m 0.25m 0.25m). The carrier gas was nitrogen, and the column was kept at 60°C for 3 minutes before rising to 220°C for 5 minutes and remaining constant at 220°C. The temperature interface was set to 280°C. Mass spectrometric analysis gave 40,800 atomic mass units (AMU) at 2,500°C. Identified compounds have peaks with greater than 90% accuracy com -pared to more than 62,000 spectral samples in the National Institute of Standard and Technology (NIST) library database and Wiley libraries.

Results:

Organoleptic properties of extracts:

The clove extracts were sticky and oily with the ethyl acetate extract being more of a sticky liquid. In addition, methanol extract appears brown while ethyl acetate extracts are light green in color (Table 1). From the result, 40g and 20g of extracts of ethyl acetate and methanol were obtained, corresponding to yields of 13.3% and 6.3%, respectively. In general, it was observed that the extracts produced by the solvents were low.

Table 1: Organoleptic properties and percentage (%) yield of methanol and ethyl acetate extraction

Sample	Extracts	WOP (g)	WOE (g)	POE (%)	COE	TOE
Cloves flower buds	Methanol	300	40	13.3	Brown	Oily
	Ethyl acetate	300	20	6.3	Light green	Oily

WOP: Weight of plant; WOE: Weight of extract; POE: Percentage of extract; COE: Color of extract; TOE: Texture of extract

Chemical compounds composition:

The chromatograms in Fig 1 show the peaks that identified compounds in methanol and ethyl acetate extracts. Nineteen compounds were identified in the extracts with the same retention time, but they had different percentage weights and peak areas. These compounds are listed in Table 2. Of these compounds, -2-methoxy-4-prop-2-enylphenol is the most abundant in the extracts (23.51%-19.03%), followed by -bisabolol (14.81%-12.54%) and acetyl-eugenol (14.11%–9.23%).

A benzene ring that is structurally common could be a relationship between the compounds, as shown in their structures in Fig 2. In addition, several minor compounds were also detected by GC-MS analysis. These compounds include methyl ester, ethyl ester, terpenes, caryophyllene, cedrenol, 1,3-benzodioxol-5-ol, tetradecanoic acid and heneicosanoic acid. They differ greatly in weight from the main components and probably play a role in the effectiveness of clove against pathogenic bacteria.

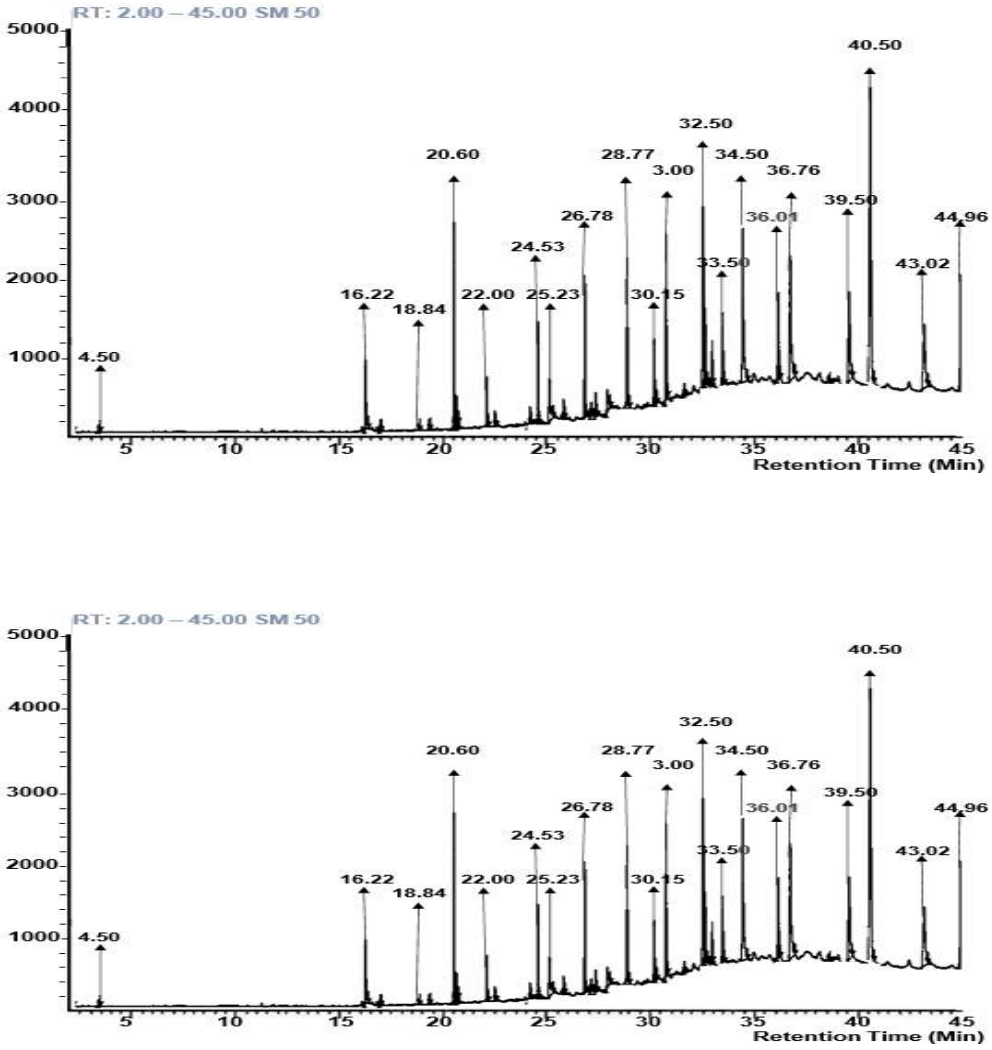
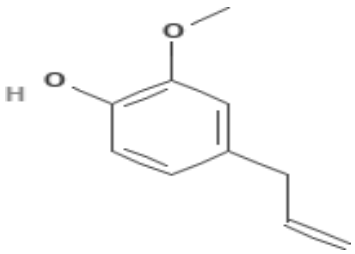


Fig 1: Peaks indicated by GC-MS analysis for identified compounds from the clove extracts

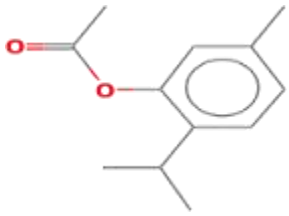
Table 2: Chemical compounds detected in the flower buds of *Syzygium aromaticum*

Retention Time	Compounds	Formula	Molecular Weight	Weight (%)		Peak Area (%)	
				a	b	a	b
4.50	1,3-Benzodioxol-5- ol	$C_7H_6O_3$	138	0.70	1.60	0.59	0.45
16.22	–2-methoxy-4- prop-2-enylphenol	$C_{10}H_{12}O_2$	352	19.03	23.51	3.20	3.11
18.84	Phenol, 5-methyl-2- (1-methylethyl)-, acetate	$C_{12}H_{16}O$	192	11.41	16.12	0.65	0.87
20.60	Acetyleugenol	$C_{12}H_{14}O_3$	206	14.11	9.23	7.93	6.27
22.00	α-Humulene	$C_{15}H_{24}$	204	4.17	2.09	2.56	2.41
24.53	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.09	1.24	1.51	2.04
25.23	1,8,11-Heptadecatriene, (Z, Z)-	$C_{17}H_{30}$	234	2.11	2.03	2.28	2.11
26.78	9,12-Octadecadienoic acid (Z, Z)	$C_{38}H_{68}O_8$	242	2.86	3.44	6.85	6.91
28.77	Humulene-1-2- epoxide	$C_5H_{29}O$	220	1.45	1.29	6.71	5.25
30.15	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	336	11.11	7.03	9.78	9.42
32.50	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.99	5.85	7.31	8.74
33.50	Cholecalciferol	$C_{27}H_{44}O$	384	2.68	3.92	4.15	5.03
34.50	Heneicosanoic acid	$C_{21}H_{42}O_2$	326	5.16	3.01	8.59	10.22
36.01	Caryophyllene	$C_{15}H_{24}$	204	6.07	3.89	6.22	2.60
36.76	Cedrenol	$C_{15}H_{24}O$	220	2.14	2.23	6.54	6.12
39.50	Geramyl-α-Terpinene	$C_{20}H_{32}$	272	2.43	2.78	4.48	4.99
40.50	α-Bisabolol	$C_{15}H_{26}O$	222	0.99	1.52	14.81	12.54
43.02	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	2.82	2.54	4.79	4.80
44.96	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	7.23	6.04	7.68	4.16

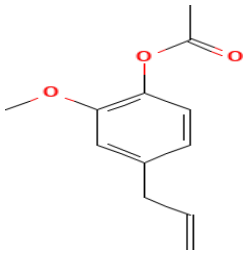
a; Methanol extract, b; ethyl acetate extract



–2-methoxy-4- prop-2-enylphenol

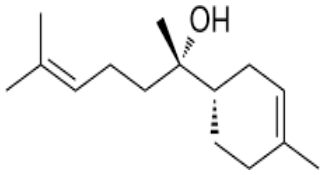


Phenol, 5-methyl-2- (1-methylethyl)-, acetate



Acetyleugenol

Eicosanoic acid, methyl ester



α-Bisabolol

Fig 2: The structure of the main compounds in clove extracts

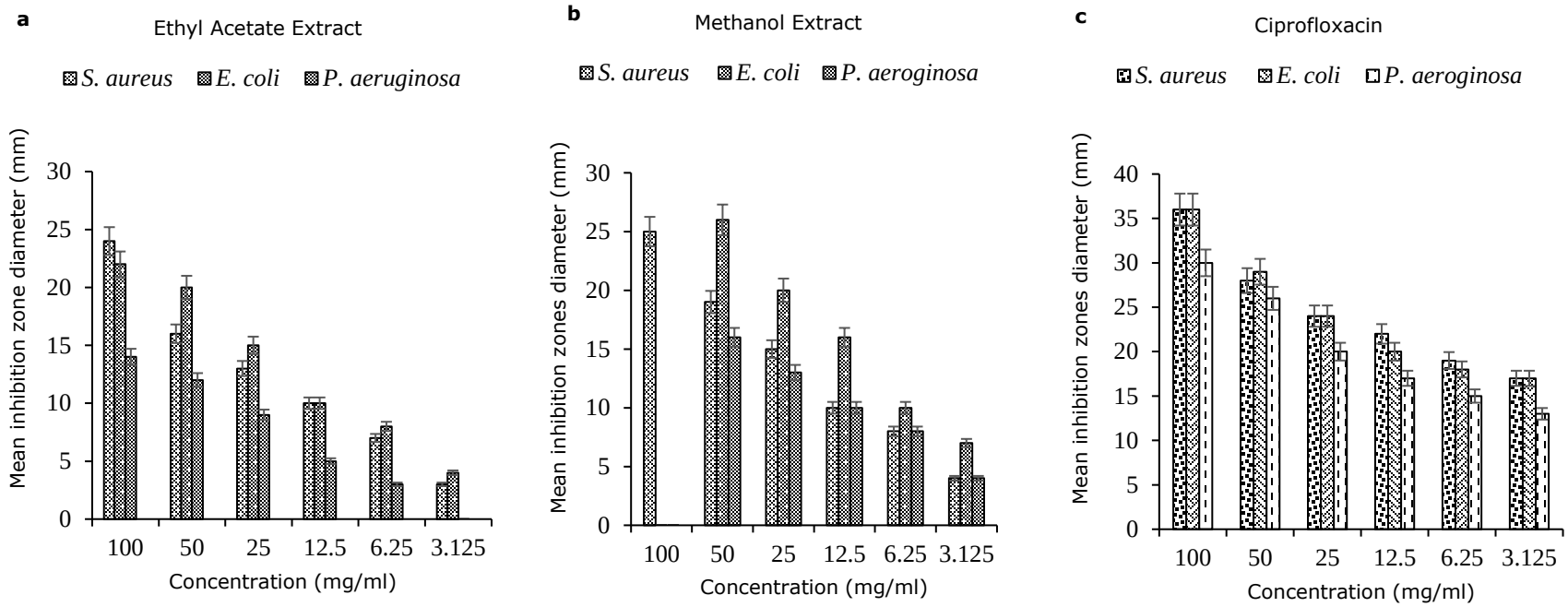


Fig 3: Antibacterial activity (inhibition zone diameter) of (**a**) ethyl acetate and (**b**) methanol extracts compared to that of (**c**) ciprofloxacin control

Results of antibacterial assay of *Syzygium aromaticum* extracts:

The antibacterial screening at 100 mg/ml of ethyl acetate extract produced inhibition zone diameter of 24mm for *S. aureus,* 22mm for *E. coli* and 14mm for *P. aeruginosa*, while methanol extract produced 25mm, 26mm and 16 mm respectively. The antibacterial assay results are presented in Fig 3. From the results, diameters of zones of inhibition increased with increasing concentrations of the extracts which compared with the zones produced by ciprofloxacin control. However, the zone of inhibition was noticeably wider with the extracts, indicating higher activity than the ciprofloxacin control.

Results of MIC and MBC of the extracts:

The MIC results showed the lowest dose that inhibited the three test organisms to be 3.125 mg/ml of ethyl acetate extract, which was below 12.5 mg/ml for the methanol extract, indicating a higher MIC of the methanol extract (Table 3). On the other hand, the test plates for MBC with 3.125 mg/ml of ethyl acetate extract showed no growth of *S. aureus* and *P. aeruginosa* but this occurred for *E. coli* at 6.25 mg/ml, while 6.25 mg/ml methanol extract represented the lowest concentration that suppressed the three test organisms. Ethyl acetate extract was found to have a more suppressive effect on the test organisms at a lower concentration of 3.125 mg/ml than methanol extract, indicating a lethal concentration that is considered the MBC (Table 4).

Discussion:

Herbal products continue to be sources of pharmacoactive properties that correlate well with drug metabolism and drug kinetics (13). Therefore, research still focuses on plants to identify bioactive compounds to treat diseases. In this study, the color differences between ethyl acetate and methanol extracts were consistent with the findings of Leonard (14). Typically, colors represent impurities, and a darker extract has a higher level of foreign matter that differs from the extract's original chemicals (15). In addition, a higher yield for methanol indicates better extraction performance, consistent with the findings of Leonard (14).

This finding was also confirmed by Temesgen et al., (16) who also had higher amount of oil extracted by methanol than by ethyl acetate. However, the observed low extract yield may be due to the non-heat method used in the extraction process. Guan et al., (17) obtained more clove extract with a higher yield than in this study due to the heat applied, confirming the effectiveness of the heating process compared to the cold maceration used in this study. This could be due to the increase in surface area that the heating process allows the solvents to penetrate the sample. This is because previous research has shown higher yields due to the heat used in the extraction process (17).

Table 3: Minimum inhibitory concentration (MIC) results of the extracts

Bacteria	Ethyl acetate extract	Methanol extract
	3.125 mg/ml	12.5 mg/ml
Staphylococcus aureus	++	+
Escherichia coli	++	+
Pseudomonas aeruginosa	++	+

++; lower concentration that showed lethal effect on the test organisms

Table 4: Minimum bactericidal concentration (MBC) results of the extracts

Bacteria	Ethyl acetate extract		Methanol extract
	6.5 mg/ml	3.125 mg/ml	12.5 mg/ml
Staphylococcus aureus		++	+
Escherichia coli	++		+
Pseudomonas aeruginosa		++	+

++; lower concentration that showed lethal effect on the test organisms

The nineteen compounds identified in this study were less than 31 and 40 chemical components identified by (18,19). The number of compounds detected is related to the quality and medicinal value of the clove extract. This investigation found –2-methoxy-4-prop-2-enylphenol as the most abundant compound in the bud of cloves. However, relatively high levels of acetyleugenol in clove buds vary, with most studies reporting high levels of eugenol (20,21). Its occurrence cannot be excluded since the cultivation area, climatic conditions and extraction methods limit the eugenol content in the clove species (22). In addition, the macerate used in this study could not adequately demonstrate the infiltration of the sample to extract eugenol due to the slow reactivity between solvent particles and the sample. This is in contrast to the heating method, which has been reported to extract eugenol from the stem, buds and leaves of cloves (21). Heating causes the movement of solvent particles to penetrate deeper into samples and extracts sensitive compounds.

The chemical components enrich the clove with antibacterial properties of medicinal importance that can be related to the medicinal values reported by traditional healers (23). Despite the complexity of organic compounds in the essential oils, clove oil is very effective and considered safe and non-harmful to health by the United States Food and Drug Administration (USFDA). Therefore, 2.5 mg/kg per day has been recommended for infected patients by the World Health Organization (WHO) due to the healing properties of the oil. Also, recent research has discovered properties in clove oils that inhibit the growth of severe acute respiratory coronavirus 2 (SARS-CoV-2) due to their ability to bind to proteases and inhibit virus replication in the host (24). This underscores the role of clove oil in treatment of diseases caused by the coronavirus, the mechanism for which is not yet fully understood.

The antibacterial activity expresses the effectiveness of the extract in inhibiting microorganisms. It was found that 100 mg/ml extracts inhibited the test organisms, consistent with the findings of Benmakhlouf et al., (20). The increased activity with increasing amount of extract compared to ciprofloxacin indicates strong inhibitory properties. In contrast to that of Moemenbellah-Fard et al., (25), who observed a decrease in activity with increasing concentration. The reason for this behavior could be the diffusion rate of the extracts. Different dilution rates limit the diffusion of the viscous liquid into the agar medium, thus inhibiting the organism.

The MIC and MBC results were inconsistent with those of Fagere and Al Magbou (26), who reported a lower value (1.50 mg/ml) than that reported in our study. As with antibacterial screening, dilution and diffusion rates could be the cause of the differences in MBC and MBC. Lower MIC and MBC values in the ethyl acetate extract indicate higher lethality compared to the methanol extract. This behavior can be related to the combined effect of lipophilic and hydrophilic compounds extracted by ethyl acetate and their synergistic effect on test organisms. Cava-Roda et al., (27) reported that the hydrophobic rings of the major and trace chemicals in cloves interact with cells and disrupt their cytoplasmic membrane.

Conclusion:

This research examined the antibacterial effect of clove buds on pathogenic microorganisms and chemical components responsible for bioactivity. GC-MS analysis revealed 2-methoxy-4-prop-2-enylphenol, phenol, 5-methyl-2-(1-methylethyl), acetate, bisabolol and acetyleugenol as the most abundant compounds in the extracts. At 100 mg/ml, the MIC of ethyl acetate and methanol extracts of the buds were almost identical. However, ethyl acetate extracts had greater bactericidal properties (MBC) and fatal effects on the test bacteria than the methanol extracts. This higher antibacterial activity indicates that ethyl acetate is a better solvent for extracting bioactive compounds from clove buds. The *invitro* efficacy of the clove buds against the test bacteria was demonstrated.

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Contributions of authors:

MA conceived, supervised and wrote the manuscript; HHJ carried out all laboratory works and collation of raw data; BTV performed all statistical analysis of the data collected; MIL analysed and discuss antibacterial results; SM collected literatures related to the plants used in the research; and UB is a chemist that discussed the compounds detected by the GC-MS analysis.

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Conflict of interest:

Authors declared no conflict of interest

References:

1. Mahomoodally, M. F., Ugurlu A., Llorent-Martínez E. J., et al. An endemic plant potentiates conventional antibiotics, inhibits clinical enzymes and induces apoptosis in breast cancer cells. Ind Crops Prod. 2020; 143: 111948. https://doi.org/10.1016/j.indcrop.2019.111948
2. Nassan, M. A., Mohamed, E. H., Abdelhafez, S., et al. Effect of clove and cinnamon extracts on experimental model of acute hematogenous pyelonephritis in albino rats: Immunopathological and antimicrobial study. Int J Immunopathol Pharmacol 2015; 28 (1): 60-68. https://doi.org/10.1177/0394632015572075
3. Batiha, G. E., Alkazmi, L. M., Wasef, L. G., et al. *Syzygium aromaticum* L. (Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 2020; (2) : 2-16 https://doi: 10.3390/biom10020202
4. Kourkouta, L., Koukourikos, K., Iliadis, C., et al. History of antibiotics. Sumerian J Med Healthcare. 2018; 1:51-54. https://www.sumerianz.com
5. Kourkouta, L., Kotsiftopoulos, C. H., Papageorgiou, M., et al. The rational use of antibiotics medicine. J Health Commun. 2017; 2(3):1-4.
6. Normark, B. H. and Normark, S. Evolution and spread of antibiotic resistance. J Inter Med. 2002; 252(2):91-106. https://doi.org/10.1046/j.1365-2796.2002.01026.x
7. Routray, A., and Mane, A. Knowledge, Attitude, and Practice (KAP) Survey on the Management of Multidrug-Resistant Gram-Negative Infections with Innovative Antibiotics: Focus on Ceftazidime–Avibactam. Cureus. 2023; 15(5): 3-14.
8. Britannica The Editors of Encyclopaedia. "Lapai" Encyclopedia Britannica, 9 Jan. 2009 https://www.britannica.com/place/Lapai (Accessed 13 June 2023)
9. Kurniawati, R. D., Martini, M., Wahyuningsih, N. E., et al. Comparison analysis of leaf and flower extraction of clove which have the potential as larvacida. Int Res J Public and Environ Health. 2022; 9(4):110. https://doi.org/10.15739/irjpeh.22.014
10. Aruljothi, S., Uma, C., Sivagurunathan, P., et al. Investigation on antibacterial activity of Carica papaya leaf extracts against wound infection-causing bacteria. Inter J Res Studies Biosci. 2014; 2 (11): 8-12.
11. Adegbenu, P. S., Aboagye, G., Amenya, P., et al. Susceptibility of bacterial and fungal isolates to spices commonly used in Ghana. Sci Afri. 2020; 9: 1-9. https://doi.org/10.1016/j.sciaf.2020.e00530
12. Ibrahim, H., Uttu, A. J., Sallau, M. S, et al. Gas chromatography–mass spectrometry (GC-MS) analysis of ethyl acetate root bark extract of *Strychnos innocua* (Delile). Beni-Suef Univ J Basic Appl Sci. 2021; 10:1-8. https://doi.org/10.1186/s43088-021-00156-1
13. Kalaimathi, R. V., Krishnaveni, K., Murugan, M., et al. ADMET informatics of Tetradecanoic acid (Myristic Acid) from ethyl acetate fraction of *Moringa oleifera* leaves. J Drug Deliv Ther. 2022; 12 (4-S): 101-111. https://doi.org/10.22270/jddt.v12i4-S.5533
14. Leonard, A. C., and Osita, O. M. Fractionation and Characterization of the Bioactive Compounds of the Extracts of Buds of *Syzygium Aromaticum*. J Chem Environ. 2022; 1(1): 56-73 https://doi.org/10.56946/jce.v1i01.28
15. Adaramola, B., Onigbinde, A. and Shokunbi, O. Physiochemical properties and antioxidant potential of *Persea americana* seed oil. Chem Int. 2016; 2 (3):168-175
16. Temesgen, S., Sasikumar, J. M., and Egigu, M. C. Effect of Extraction Solvents on Total Polyphenolic Content and Antioxidant Capacity of *Syzygium aromaticum* L. Flower Bud from Ethiopia. Bio Med Res Int. 2022; 1-9. https://doi.org/10.1155/2022/4568944
17. Guan, W., Li, S., Yan, R., et al. Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food Chem. 2007; 101(4): 1558 -1564. https://doi.org/10.1016/j.foodchem.2006.04.009
18. Bhuiyan, M. N. I., Begum, J., Nandi, N. C., and Akter, F. Constituents of the essential oil from leaves and buds of clove (*Syzygium caryophyllatum* (L.) Alston). Afr J Plant Sci. 2010; 4(11): 451-454. http://www.academicjournals.org/ajps
19. Chen, Y., Xu, F., Pang, M., et al. Microwave-assisted hydrodistillation extraction based on microwave-assisted preparation of deep eutectic solvents coupled with GC-MS for analysis of essential oils from clove buds. Sustain Chem Pharm. 2022; 27: 100695. https://doi.org/10.1016/j.scp.2022.100695
20. Benmakhlouf, Z., Benserradj, O., and Kellab, R. Identification of phytochemical constituents of *Syzygium aromaticum* L. using gas chromatography coupled with mass spectrometry and evaluation of antimicrobial activity. Biodiversitas J Biolog Diversity. 2022; 23(5): 2586-2593. https://doi.org/10.13057/biodiv/d230540
21. Rajalekshmy, V. S., and Manimekalai, V. Phytochemical Analysis of the Flower Buds, Pedicels and Leaves of *Syzygium aromaticum* (L.) Merril and Perry. Technol Innov Pharm Res. 2021; 5: 111-119. https://doi: 10.9734/bpi/tipr/v5/9672D
22. Teles, A. M., Silva-Silva, J. V., Fernandes, J. M. P., et al. GC-MS characterization of antibacterial, antioxidant, and antitrypanosomal activity of *Syzygium aromaticum* essential oil and eugenol. Evidence-Based Complem Altern Med. 2021; 1-12. https://doi.org/10.1155/2021/6663255
23. Abdel-Tawwab, M., Adeshina, I., Jenyo-Oni, A., et al. Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.) to dietary clove basil, *Ocimum gratissimum* leaf extract and its susceptibility to *Listeria monocytogenes* infection. Fish Shellfish Immunol. 2018; 78:346-54. https://doi.org/10.1016/j.fsi.2018.04.057
24. Chandra Manivannan, A., Malaisamy, A., Eswaran, M., et al. Evaluation of clove phytochemicals as potential antiviral drug candidates targeting SARS-CoV-2 main protease: Computational Docking, molecular dynamics simulation, and pharmacokinetic profiling. Front Mol Biosci. 2022; 9 (604): 1-12. https://doi.org/10.3389/fmolb.2022.918101
25. Moemenbellah-Fard, M. D., Abdollahi, A., Ghanbariasad, A., et al. Antibacterial and leishmanicidal activities of *Syzygium aromaticum* essential oil versus its major ingredient, eugenol. Flavour Fragr J. 2020; 35(5): 534-540. https://doi.org/10.1002/ffj.3595
26. Fagere, Z. O., and Al Magbou, A. Z. Antibacterial activity of clove oil against some microorganisms at Khartoum State, Sudan. Adv Med Plant Res. 2016; 4(4): 122-128.
27. Cava-Roda, R., Taboada-Rodríguez, A., López-Gómez, A., Martínez-Hernández, G. B., and Marín-Iniesta, F. Synergistic antimicrobial activities of combinations of vanillin and essential oils of cinnamon bark, cinnamon leaves, and cloves. Foods. 2021; 10(6): 1406. https://doi.org/10.3390/foods10061406

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Short Communication **Open Access**

COVID-19 and Dengue co-infection in paediatric patients: An endemic in a pandemic

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Abstract:

Background: The clinical presentation and outcome of COVID-19 are likely to be complicated by co-infection with other endemic viruses such as Dengue. This study aimed to investigate the occurrence of co-infections of SARS-CoV-2 and Dengue viruses in paediatric patients from Osmania General Hospital, Hyderabad, Telangana, during the COVID-19 pandemic.
Methodology: This was a cross-sectional study of 420 paediatric patients (aged 5-17 years) with febrile illness, consecutively recruited from the hospital from June to November 2021. Serum samples were collected and tested for SARS-CoV-2 antibodies, and COVID-19 positive samples were further analysed for Dengue IgM antibodies by enzyme linked immunosorbent assay (ELISA).
Results: Of the 420 patients, serum samples of 109 (26.0%) were reactive for SARS-CoV-2 antibodies. Of these, 13 were reactive for Dengue IgM antibodies, giving a co-infection rate of 3.1% (1.9% females and 1.2% males). The three most common symptoms in the co-infected patients were joint ache (myalgia) in 53.8% (75.0% in females, 20.0% in males, p=0.1026), fever in 46.2% (50.0% in females, 40.0% in males, p=1.000), and rash in 46.2% (62.5% in females, 20.0% in males, p=0.2657).
Conclusion: These findings suggest that paediatric patients with COVID-19 may be susceptible to Dengue. Understanding the presence of multiple viral infections in paediatric patients is crucial for accurate diagnosis, management, and prognosis. Further research is needed to explore the potential synergistic mechanisms of these co-infections.

Keywords: SARS-CoV-2, COVID-19, Dengue, co-infection, paediatrics, antibodies, ELISA.

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Co-infection COVID-19 et dengue chez les patients pédiatriques: une endémie dans une pandémie

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Résumé:

Contexte: La présentation clinique et l'issue de la COVID-19 sont susceptibles d'être compliquées par une co-infection avec d'autres virus endémiques tels que la dengue. Cette étude visait à étudier la survenue de co-infections par les virus du SRAS-CoV-2 et de la Dengue chez des patients pédiatriques de l'Hôpital Général d'Osmania, Hyderabad, Telangana, pendant la pandémie de COVID-19.
Méthodologie: Il s'agissait d'une étude transversale de 420 patients pédiatriques (âgés de 5 à 17 ans) atteints de maladie fébrile, recrutés consécutivement à l'hôpital de Juin à Novembre 2021. Des échantillons de sérum ont été prélevés et testés pour les anticorps anti-SARS-CoV-2, et Les échantillons positifs au COVID-19 ont été analysés plus en détail pour les anticorps IgM de la dengue par dosage immuno-enzymatique (ELISA).
Résultats: Sur les 420 patients, des échantillons de sérum de 109 (26,0%) étaient réactifs pour les anticorps SARS-CoV-2. Parmi ceux-ci, 13 étaient réactifs aux anticorps IgM de la dengue, soit un taux de co-infection de 3,1% (1,9% de femmes et 1,2% d'hommes). Les trois symptômes les plus fréquents chez les patients co-infectés étaient les douleurs articulaires (myalgies) chez 53,8% (75,0% chez les femmes, 20,0% chez les hommes,

p=0,1026), la fièvre chez 46,2% (50,0% chez les femmes, 40,0% chez les hommes, *p*=1,000) et éruption cutanée chez 46,2% (62,5% chez les femmes, 20,0% chez les hommes, *p*=0,2657).
Conclusion: Ces résultats suggèrent que les patients pédiatriques atteints de COVID-19 peuvent être sensibles à la dengue. Comprendre la présence d'infections virales multiples chez les patients pédiatriques est crucial pour un diagnostic, une prise en charge et un pronostic précis. Des recherches supplémentaires sont nécessaires pour explorer les mécanismes synergiques potentiels de ces co-infections.

Mots clés: SARS-CoV-2, COVID-19, Dengue, co-infection, pédiatrie, anticorps, ELISA

Introduction:

The COVID-19 pandemic highlighted the significance of understanding the clinical implications and dynamics of co-infections, particularly in the paediatric age group. Paediatric patients with SARS-CoV-2 infections often present with acute sickness and hyperinflammatory syndrome that can lead to severe outcomes such as multiorgan failure and shock (1). Simultaneously, Dengue virus infections continue to pose a major public health challenge, with millions of annual cases reported worldwide (2).

The coexistence of Dengue epidemics in tropical regions with the SARS-CoV-2 pandemic raises concerns due to the shared clinical features and potential for missed cases, which can have fatal consequences (3). The range of clinical symptoms associated with SARS-CoV-2 infections varies from mild respiratory symptoms resembling a common cold to life-threatening conditions such as bronchiolitis, pneumonia, acute respiratory distress syndrome (ARDS), inflammatory syndrome, and multi-organ failure. In this context, it becomes crucial to understand the dynamics of co-infection between Dengue and COVID-19 to accurately assess the clinical impact and enable early interventions (4,5).

However, differentiating Dengue and COVID-19 in endemic regions during the pandemic poses' challenges, particularly in the early stages of infection when symptoms may overlap (6,7). The simultaneous peak in Dengue and SARS-CoV-2 infections observed in the year 2020 further strained healthcare systems, with the implementation of lockdowns and social segregation policies exacerbating the spread of Dengue (1,8). Therefore, there is a pressing need to identify clinical and laboratory factors that can predict co-infection in paediatric COVID-19 patients.

Early identification of co-infections is essential to guide appropriate antibiotic selection and provide timely supportive care. This study aims to address this gap by analysing the range of co-infections in paediatric COVID-19 patients and identifying factors that can aid in the prediction of co-infection, ultimately facilitating early interventions and improved patient management.

Materials and method:

Study design and ethical approval:

A cross sectional study was conducted at the Osmania General Hospital, Hyderabad, Telangana, from June to November 2021. The study protocol was approved by the ethical committee (IEC/OMC/M.N.49(Acad)/60), and informed consent was obtained from the parents of all participating paediatric patients.

Study participants and data/sample collection:

Patients aged 5-17 years presenting with symptoms of COVID-19 were consecutively recruited and included in the study. A structured questionnaire was used to collect demographic details and record clinical symptoms. Blood samples were collected from each participant, serum was separated and stored at -20 ºC until further processing.

Serological testing:

The serum samples were tested for presence of SARS-CoV-2 antibodies using the Wantai SARS-CoV-2 Ab ELISA kit (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China). Subsequently, positive samples were tested for Dengue IgM antibodies using the ICMR-NIV MAC ELISA kit (ICMR-National Institute of Virology, Pune, India).

For SARS-CoV-2 antibody detection, the assay was performed by adding negative and positive controls, as well as the serum samples, to microwells coated with SARS-CoV-2 antigen. HRP conjugate was added, followed by the addition of TMB substrate, which was then incubated in a dark room. The reaction was stopped by the addition of H_2SO_4, and the absorbance value at 450nm was measured to interpret the results. An absorbance value above the cut-off was considered positive, indicating the presence of antibodies to SARS-CoV-2.

For Dengue IgM detection, the assay utilized a capture ELISA approach. During incubation, IgM in the patient's serum were captured by anti-human IgM. After washing, DEN antigen was added and incubated. Any unbound antigen was removed by further washing, followed by the addition of DEN monoclonal antibody and Avidin HRP. Chromogenic substrate was added and the reaction was stopped using 1N H_2SO_4. The intensity of the resulting

color was measured at 450nm. The results of the serological testing were analysed based on absorbance values and interpreted according to predetermined cut-off values for each assay.

Statistical analysis of data:

Descriptive statistics were used to summarize the demographic and clinical data. Chi-Square (or Fisher Exact) test was used to compare categorical variables and the level of significance was $p<0.05$.

Results:

During the study period, serum samples were collected from 420 paediatric patients with symptoms of COVID-19 out of which 109 (26.0%) were reactive to SARS-CoV-2 antibodies. Out of the 109 seropositive patients, 54 (49.5%) were males and 55 (50.5%) were females. The age range of the seropositive patients was 5-17 years. Twenty three of 55 (41.8%) female and 24 of 54 (44.4%) male patients had fever of more than 38ºC.

A total of 13 among the 109 seropositive samples were reactive to Dengue IgM, giving co-infection of Dengue and SARS-CoV2 rate of 3.1% (13/420), with 8 (1.9%) females and 5 (1.2%) males. The clinical presentations of the 13 patients with co-infection of SARS-COV-2 and Dengue are shown in Table 1, with the three most common symptoms being joint ache (myalgia) in 53.8% (75.0% in females, 20.0% in males, p=0.1026), fever in 46.2% (50.0% in females, 40.0% in males, p= 1.000), and rash in 46.2% (62.5% in females, 20.0% in males, p=0.2657).

Discussion:

The co-infection of SARS-CoV-2 and Dengue in paediatric patients is a matter of concern due to the overlapping clinical features and potential complications associated with both viral infections. Our study, which included 420 symptomatic paediatric patients, showed 26.0% SARS-CoV-2 seropositivity and 3.1% co-infection of COVID-19 and Dengue. The clinical similarities between COVID-19 and Dengue pose a challenge in distinguishing between the two infections. There is significant overlap in the clinical symptoms of Dengue and COVID-19 including fever, myalgia, headache, cough, dyspnoea, vomiting, abdominal pain, and skin rashes (9-11). In our study, we observed similar symptoms in the co-infected patients.

There was a slightly higher prevalence of co-infection in females, with 8 (1.9%) females and 5 males (1.2%) showing co-infection. The presence of high-grade fever was more frequent in females (50.0%, 4/8) compared to males (40.0%, 2/5), indicating a potential gender difference in symptom severity, although the small sample size here makes this observation of little importance, but this finding is consistent with previous studies reporting mild to moderate illness in children with COVID-19 (12).

Table 1: Clinical manifestations of COVID-19 and Dengue co-infection in paediatric patients in Osmania General Hospital, Hyderabad, Telangana, India

Clinical symptoms	Female (%) (n=8)	Male (%) (n=5)	Total (%) (n=13)	OR (95% CI)	*p* value
Fever (≥38°C)	4 (50.0)	2 (40.0)	6 (46.2)	1.50 (0.1559-14.428)	1.000
Sore throat	3 (37.5)	0	3 (23.1)		
Runny nose (rhinorrhea)	2 (25.0)	1 (20.0)	3 (23.1)		
Cough	3 (37.5)	1 (20.0)	4 (30.8)		
Shortness of breath (dyspnea)	0	0	0		
Chills	2 (25.0)	2 (40.0)	4 (30.8)		
Vomiting	2 (25.0)	1 (20.0)	3 (23.1)		
Nausea	1 (12.5)	1 (20.0)	2 (15.4)		
Diarrhea	1 ((12.5)	3 (60.0)	4 (30.8)		
Headache	1 (12.5)	4 (80.0)	5 (38.5)		
Rash	5 (62.5)	1 (20.0)	6 (46.2)	6.667 (0.4863-91.39)	0.2657
Conjunctivitis	2 (25.0)	0	2 (15.2)		
Muscle aches	1 (12.5)	0	1 (7.7)		
Joint ache (myalgia)	6 (75.0)	1 (20.0)	7 (53.8)	12.00 (0.7952-181.1)	0.1026
Loss of appetite	1 (12.5)	0	1 (7.7)		

Interestingly, the co-infected patients did not exhibit symptoms of shortness of breath, which is commonly associated with severe COVID-19 cases. This suggests that the presence of Dengue antibodies might have a mitigating effect on the severity of Dengue symptoms, as hypothesized by Namita Ravikumar et al., (7). Studies by Ratageri et al., (11) and Khalil et al., (12) reported cases of co-infection between Dengue and SARS-CoV-2, with varying symptom severity and outcomes in paediatric patients. Additionally, Ghosh et al., (4) documented multiple cases of co-infection of COVID-19 with various diseases in the paediatric population, highlighting the need for vigilance in diagnosing and managing co-infections. Co-epidemics of Dengue and COVID-19 have been reported in Brazil and other regions, indicating the potential for simultaneous outbreaks and increased burden on healthcare systems (13-17). However, studies in adults, such as the one by Joubert et al., (14) suggest that severe dengue cases may not be more symptomatic than mild to moderate COVID-19 cases.

The limitations of our study include the relatively small sample size and its single-centre nature (18-20). However, the study highlights the co-infection of SARS-CoV-2 and Dengue in paediatric patients, emphasizing the need for awareness and early detection of these dual infections. The overlapping clinical features and potential complications necessitate careful evaluation and management of co-infected cases.

Further research with larger sample size and multi-centre collaborations are needed to validate our findings and gain better understanding of the clinical impact and outcomes associated with co-infection of SARS-CoV-2 and Dengue in paediatric patients. There is also the need to explore the mechanisms underlying the interaction between the two viruses and to develop effective strategies for diagnosis, treatment, and prevention of co-infection in paediatric populations.

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Contributions of authors:

PS was involved in the concept and design of the study; SW and RV were involved with data collection and serological testing; SW, SM and MSF prepared the first draft of the manuscript; RVS reviewed and edited the manuscript. All the authors read and approved the final manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

References:

1. Ferdous, A., Hossain, M. M., and Afrin, M. Severe Dengue with Multisystem Inflammatory Syndrome in Children Due to COVID-19: A Co-infection Case Series. Cureus. 2021; 13 (11): e19516. doi: 10.7759/cureus.19516.
2. Wiyono, L., Rocha, I. C. N., Cedeño, T. D. D., Miranda, A. V., and Lucero-Prisno Iii, D. E. Dengue and COVID-19 infections in the ASEAN region: a concurrent outbreak of viral diseases. Epidemiol Health. 2021; 43: e2021070. doi: 10.4178/epih.e2021070.
3. Tiwari, L., Shekhar, S., Bansal, A., and Kumar, P. COVID-19 with dengue shock syndrome in a child: coinfection or cross-reactivity? BMJ Case Reports CP. 2020; 13(12): e239315.
4. Ghosh, U., Baidya, M., and Sultana, A. Co-infections and COVID-19 in children: an observational study. Int J Innov Sci Res Technol. 2020; 5: 343-347.
5. Raychaudhuri, D., Sarkar, M., Roy, A., et al. COVID-19 and Co-infection in children: The Indian perspectives. J Trop Pediatr. 2021; 67(4): fmab073.
6. Garg, S., and Meena, S. Symptomatic similarity, co-infection and cross-reactivity in dengue and Covid-19 disease. Int J Mosq Res. 2021; 8 (3): 64-68.
7. Ravikumar, N., Randhawa, M. S., Nallasamy, K., et al. Impact of recent SARS-CoV-2 infection on the course and severity of dengue in children: a prospective observational study from North India. Am J Trop Med Hyg Title. 2021; 105 (3): 751.
8. Ravikumar, N., Dalla Pozza, L., Wäckerlin, R., and Schranz, D. Coinfection of SARS-CoV-2 and dengue virus: A case series from Switzerland. Pediatr Infect Dis J. 2021; 40(12): e497-e500.
9. Henrina, H., Putra, I., Lawrensia, S., Handoyono, Q., and Alius, C. Coronavirus disease of: a mimicker of dengue infection? SN Compr Clin Med. 2019-2020; 13: 1-11.
10. Somasetia, D. H., Malahayati, T. T., Andriyani, F. M., Setiabudi, D., and Nataprawira, H. M. A fatal course of multiple inflammatory syndromes in children coinfection with dengue. A case report from Indonesia. IDCases. 2020; 22: e01002.
11. Ratageri, V. H., Pawar, G. R., Nikhil, G., and George, S. S. Co-Infection of Dengue Fever with COVID-19 in a Child with MIS-C. Indian J Pediatr. 2021; 88 (5): 485. doi: 10.1007/s12098-021-03701-z.
12. Khalil, A., Badr, B., Wright, H., Talo, M., and Atteiya, M. Dengue Fever and COVID-19 Co-Infection at the Emergency Department of a Tertiary Care Hospital in Jeddah, Saudi Arabia. Eur J Case Rep Intern Med. 2020; 7(12): 002167. doi: 10.12890/2020_002167.
13. Magalhaes, T., Chalegre, K. D. M., Braga, C., and Foy, B. D. The endless challenges of arboviral diseases in Brazil. Trop Med Infect Dis. 2020; 5: 75.
14. Joubert, A., Andry, F., Bertolotti, A., et al. Distinguishing non severe cases of dengue from

COVID-19 in the context of co-epidemics: a cohort study in a SARS-CoV-2 testing center on Reunion Island. PLoS Negl Trop Dis. 2021; 15: e0008879

15. Verduyn, M., Allou, N., Gazaille, V., et al. Co-infection of dengue and COVID-19: A case report. PLoS Negl Trop Dis. 2020; 14(8): e0008476. doi: 10.1371/journal.pntd.0008476.
16. Cardona-Ospina, J. A., Henao-SanMartin, V., and Paniz-Mondolfi, A. E. SARS-CoV-2, dengue virus, and overlapping epidemics: a double concern for Dengue-endemic countries. Ann Clin Microbiol Antimicrob. 2020; 19(1): 1-3.
17. Joubert, J., Preller, R., and van Vuuren, C. Severe dengue versus mild COVID-19–Two worlds apart. J Infect. 2021; 82(2): e6-e7.
18. Hertzog, M. A. Considerations in determining sample size for pilot studies. Res Nurs Health. 2008: 31(2): 180-191.
19. Gupta, S. Single-center studies: a matter of concern. Indian Journal of Critical Care Medicine. 2011; 15 (1): 3-4.
20. Sedgwick, P. Sample size in observational studies. BMJ, 2014; 348: g1176.

Liwata et al. *Afr. J. Clin. Exper. Microbiol.* 2023; 24 (4): 403 - 407 https://www.afrjcem.org

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Short Communication **Open Access**

In vitro antimicrobial activity of Mathesia® on bacterial isolates of wound infections in University Clinics and Hospital Centre of Mont Amba, Kinshasa, Democratic Republic of the Congo

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Abstract:

Background: Mathesia is a hydro-alcoholic emulsion, colourless and somewhat viscous, based on extracts of medicinal plants and containing saponins, polyphenols, tannins, and reducing sugars. In this study, we proposed to carry out an *in vitro* study of the antibacterial activity of phytomedicine Mathesia on strains of bacteria isolated from diabetic foot ulcers and chronic wounds of patients in care in the University Clinics and the Hospital Centre of Mont Amba in Kinshasa, Democratic Republic of the Congo.
Methodology: This *in vitro* study was carried out in the bacteriology laboratory of the Higher Institute of Medical Technology of Kinshasa from February to June 2022. The Kirby-Bauer disc diffusion method on Mueller-Hinton agar was used for antibacterial assay of different concentrations of Mathesia on 7 different Gram-positive and Gram-negative bacterial species isolated from chronic wounds. The minimum inhibitory concentration (MIC) of Mathesia for the isolates was determined by broth dilution method on Mueller Hinton broth media.
Results: The results from this study showed that Mathesia has an inhibitory effect upon the 7 bacterial species at MIC value of less than 100 µg/ml. The lowest MIC value of 1.95 µg/ml was obtained against *Staphylococcus aureus, Staphylococcus epidermidis* and *Proteus* species.
Conclusion: The results obtained in this study corroborate previous studies which demonstrated effectiveness of Mathesia on *Escherichia coli, Streptococcus pyogenes* and *Aspergillus* species. This activity could also be justified by the presence of phenolic acids, tannins and flavonoids which possess antibacterial properties.

Keywords: Phytomedicine; Mathesia; antibacterial activity; wound infection; multi-drug resistant bacteria

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Activité antimicrobienne *in vitro* de Mathesia® sur des isolats bactériens d'infections de plaies dans les Cliniques Universitaires et Centre Hospitalier du Mont Amba, Kinshasa, République Démocratique du Congo

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Résumé:

Contexte: Mathesia est une émulsion hydro-alcoolique, incolore et quelque peu visqueuse, à base d'extraits de plantes médicinales et contenant des saponines, des polyphénols, des tanins et des sucres réducteurs. Dans cette étude, nous avons proposé de réaliser une étude in vitro de l'activité antibactérienne de phytomédicament Mathesia sur des souches de bactéries isolées d'ulcères du pied diabétique et de plaies chroniques de patients pris en charge aux Cliniques Universitaires et au Centre Hospitalier du Mont Amba à Kinshasa, République Démocratique du Congo.
Méthodologie: Cette étude *in vitro* a été réalisée au laboratoire de bactériologie de l'Institut Supérieur des Technologies Médicales de Kinshasa de Février à Juin 2022. La méthode de diffusion sur disque Kirby-Bauer sur gélose Mueller-Hinton a été utilisée pour le dosage antibactérien de différentes concentrations de Mathesia sur 7 espèces bactériennes Gram-positives et Gram-négatives différentes isolées de plaies chroniques. La concentration minimale inhibitrice (CMI) de Mathesia pour les isolats a été déterminée par la méthode de dilution en bouillon sur milieu de bouillon Mueller Hinton.
Résultats: Les résultats de cette étude ont montré que Mathesia a un effet inhibiteur sur les 7 espèces bactériennes à une valeur CMI inférieure à 100 µg/ml. La valeur CMI la plus basse de 1,95 µg/ml a été obtenue contre les espèces *Staphylococcus aureus, Staphylococcus epidermidis* et *Proteus*.
Conclusion: Les résultats obtenus dans cette étude corroborent des études antérieures qui démontraient l'efficacité de Mathesia sur les espèces *Escherichia coli, Streptococcus pyogenes* et *Aspergillus*. Cette activité pourrait également être justifiée par la présence d'acides phénoliques, de tanins et de flavonoïdes possédant des propriétés antibactériennes.

Mots-clés: Phytomédicament; Mathesia; activité antibactérienne; infection de la plaie; bactéries multirésistantes

Introduction:

A wound is a physical injury involving a break in the continuity of the skin (1). The exposed subcutaneous tissues provide a favorable substrate of growth of a wide variety of micro-organisms; and if the tissues involved are devitalized and the host immune answering is compromised, the conditions become optimal for microbial growth (2). Indeed, the host immune answering process is an important factor in the occurrence of infection (3). Infection of a wound refers to a deposition and multiplication of bacteria on the tissue with reactions that can be classic signs like redness, pain, swelling and fever (4,5). The evolution of a wound to an infected state is dependent on a multitude of microbes and some factors include the type, location, and the depth of the wound, the extent of exogenous contamination, the general health of the wounded person, the immune status of the host, the microbial load, and the virulence combined with the types of micro-organism present (2).

The infection of the majority of wounds is polymicrobial with both aerobic and anaerobic bacteria. Aerobes often include *Sta phylococcus aureus*, *Pseudomonas* and beta-haemolytic streptococcus which are frequently cited as a cause of prolonged wound healing (6-9). Tengrove et al., (10) reported that wound infection is not caused by a single micro-organism but by typical organisms including *Streptococcus* spp, *S. aureus, Pseudomonas* spp; *Escherichia coli, Klebsiella* spp, *Proteus* spp, *Bacteroides fragilis, Clostridium* spp, *Candida* spp and *Aspergillus*. These microorganisms are important in terms of mortality, morbidity, long hospital stay and delayed scarring from infections caused by them (10). Wound infections caused by multi-antibiotic resistant microbes are also costly to treat (11).

Another category of wounds that are more prone to severe infection or complicated by gangrene are the foot ulcers of diabetics. Indeed, more than a third of persons with diabetics develop foot ulcers, and half of these present with infections that result in amputation of foot or digit (12). Bacteria involved in such diabetic ulcers are both Gram-positive and Gram-negative bacteria. Of the Gram-positive bacteria, streptococcus (*S. agalactiae, S., pyogenes, S. mitis*), *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* are frequently involved while among the Gram-negatives, members of the Enterobacterales such as *Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis*, *Citrobacter,* as well as *Pseudomonas* and *Prevotella* spp frequently predominate (13).

Many studies have reported that alcoholic extracts of several plants are active against multi-drug resistant bacteria isolated from infected wounds, with minimum inhibitory concentrations ranging from 50 to 60 mg /ml (14-16). One *in vitro* efficacy study of a phytomedicine plant, Mathesia on multidrug resistant strains of tubercle bacillus (Koch's bacillus) by Kabedi et al., (17), showed that Mathesia was active against all the 33 strains isolated from TB patients, with a minimum inhibitory concentration of 30 µg/ml.

Mathesia is a hydro-alcoholic emulsion, colourless and viscous extracts of medicinal plants, containing saponins, polyphenols, tannins, and reducing sugars (18). This phytomedicine was developed by the team of Professor Mulenga Mbombo at the Faculty of Sciences of the University of Kinshasa in the Democratic Republic of Congo. We propose to conduct an *in vitro* study of the antibacterial activity of Mathesia on wild strains of bacteria isolated from diabetic foot ulcers and chronic wounds of patients hospitalized at the Cliniques Universities of Kinshasa and at the Mont Amba Hospital in the Democratic Republic of Congo.

Materials and method:

Study location and period:

The study was carried out at the bacteriology laboratory of the Higher Institute of Medical Technology of Kinshasa from February to June 2022.

Bacterial isolates:

The study was carried out on a collection of seven bacteria species isolated from different wounds of patients. These included 5 isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*, 6 isolates of *Escherichia coli*, and 1 isolate each of *Proteus* spp*, Klebsiella* spp*, Citrobacter* spp*,* and *Pseudomonas aeruginosa*.

Phytomedicinal plant Mathesia®:

Mathesia® was obtained from the Industrial and Technical Group (GITCO), in Kinshasa, DRC. The solution of Mathesia was prepared for different amounts of lyophilized Mathesia® with 14 different dilutions, ranging from 1000 µg/ml up to 0.121 µg/ml.

Determination of antibacterial activity of Mathesia by disc diffusion method:

The agar disc diffusion method was used to determine the antibacterial activity of Mathesia® extracts on the bacterial isolates. An inoculum of the overnight (~18 hours) culture of each bacteria isolate was made and standardized to match the turbidity of 0.5 McFarland standard which contains ~10^8 colony-forming units per milliliter (CFU/ml). The inoculum was spread on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 10 µl of Mathesia extract solution (1000 µg/ml). Prepared gentamicin (10 µg/ml) and norfloxacin (20 µg/ml) discs were used as reference discs.

The Mathesia and reference discs were placed on the inoculated plates and allowed to dry for 30 mins. The plates were then incubated aerobically at 37°C for 24hrs. The diameters of the inhibition zones were measured in millimeters (19), and the inhibition zones produced by Mathesia discs were compared with those of gentamicin and norfloxacin reference discs. The procedure was performed in triplicate and the mean diameter of inhibition zone (in mm) produced by Mathesia and the reference antibiotic discs was calculated for each bacterial isolate.

Determination of minimum inhibitory concentration (MIC) of Mathesia:

The minimum inhibitory concentration was determined by broth micro-dilution method as previously described (20-22). The inocula of the bacterial isolates were prepared from 24 hours old broth cultures. The absorbance was read at 600 nm and adjusted with sterile physiological solution to match that of 0.5 McFarland standard solution. From the prepared microbial solutions, other dilutions with sterile physiological solution were prepared to give a final concentration of 10^6 CFU/ml.

Stock solutions of the extracts were prepared in 0.1% (v/v) aqueous tween 80 (Fisher chemicals) at concentrations of 1 mg/ml. The two-fold serial dilutions in concentrations of the extracts were prepared in Mueller Hinton Broth (MHB), to give final concentrations ranging from 1000 to 0.121 µg/ml. An aliquot (10 µL) of a 10^6 CFU/mL overnight culture was added to wells of a sterile 96-well micro-plate titer. The positive control wells contained MHB + bacteria suspension without plant extract while negative control wells contained MHB only.

The MIC was determined as the lowest plant extract concentration at which no growth was observed after 24 hours. The viability of the bacterial growth was evaluated by the MTT (30 µl tetrazole in 0.01% aqueous solution) assay. For MBC determination, 10 µl was taken from each well of complete inhibition of bacterial growth after incubation and spot inoculated on freshly prepared MHB and incubated for 72 hours at 37°C.

Results:

Antibacterial effects of Mathesia by disc diffusion method on the bacterial isolates:

The sensitivity test result is shown in Table 1. According to Berche et al., (23), an extract is considered to have *in vitro* antibacterial effects when it produces an inhibition zone diameter ≥ 10 mm, hence Mathesia is active against all the tested bacterial isolates. The most susceptible isolates (measured by the mean diameter of inhibition zone) in

Table 1: Mean diameter of inhibition zone of Mathesia and reference antibiotics (gentamicin and norfloxacin) against the bacterial isolates

Bacteria isolates	**Mean diameter of inhibition zone (mm)**		
	Mathesia (1000 µg/ml)	**Gentamicin (10 µg/ml)**	**Norfloxacin (20 µg/ml)**
Staphylococcus aureus (n=5)	27.2±0.2[a]	18.22±0.608[b]	6.42±0.424[c]
Staphylococcus epidermidis (n=5)	26.08±0.46[a]	23.1±0.16[b]	7.16±0.27[c]
Escherichia coli (n=6)	21.7±0.4[a]	24.03±0.51[b]	17.5±0.67[c]
Klebsiella spp (n=3)	22.0± 0.0	20.0±0.0	17.5±0.0
Proteus spp (n=3)	23.0±0.0	21.0±0.0	20.7±0.0
Citrobacter spp (n=3)	19.0±0.0	20.5±0.0	19.5±0.0
Pseudomonas aeruginosa (n=3)	18.0±0.0	19.0±0.0	21.0±0.0

a, b, c: statistically significant difference from results of multiple comparisons tests (least significant difference test) between the bacterial isolates

Table 2: Minimum inhibitory concentrations of Mathesia against the bacterial isolates

Bacterial isolates	**Concentrations of Mathesia (µg/ml)**														**MIC value (µg/ml)**
	1000	500	250	125	62.5	31.25	15.62	7.81	3.9	1.95	0.97	0.485	0.242	0.121	
S. aureus (n=5)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
S. epidermidis (n=5)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
Proteus spp (n=1)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
Klebsiella spp (n=1)	+	+	+	+	+	+	+	+	+	-	-	-	-	-	3.9
Escherichia coli (n=6)	+	+	+	+	+	+	+	+	-	-	-	-	-	-	7.81
Citrobacter spp (n=1)	+	+	+	+	+	+	+	-	-	-	-	-	-	-	15.62
P. aeruginosa (n=1)	+	+	+	+	+	+	-	-	-	-	-	-	-	-	31.25

+ = growth; - = No growth; MIC = Minimum inhibitory concentration.

descending order are *S. aureus* (27.2±1.2mm), *S. epidermidis* (26.08±0.46mm), *Proteus* spp (23±0.0mm), *Klebsiella* spp (22±0.0mm), *E. coli* (21.7±0.4mm), *Citrobacter* sp (19±0.0mm) and *P. aeruginosa* (18±0.0mm).

Compared to the reference antibiotics (gentamicin and norfloxacin) used, Mathesia demonstrated significantly higher antibacterial activity against *S. aureus, S. epidermidis, Proteus* spp, and *Klebsiella* spp (Table 1).

Minimum inhibitory concentration of Mathesia against the bacterial isolates:

The results of broth dilution MIC test of the different concentrations of Mathesia (1000 µg/ml to 0.121 µg/ml) on the bacterial isolates is presented in Table 2. The MIC of Mathesia was lowest (1.95 µg/ml) for *S. aureus, S. epidermidis* and *Proteus* spp, and highest for *Pseudomonas aeruginosa* (31.25 µg/ml).

Discussion:

From the results obtained in this study, it was clearly noticed that the phytomedicine Mathesia had inhibitory effects on the seven bacterial isolates tested, which was explained by the MIC values obtained that were less than 100 µg/ml for all the seven isolates (22,24). The lowest MIC value (1.95 µg/ml) was recorded for *S. aureus, S. epidermidis* and *Proteus* spp. The results obtained in this study corroborated our past research which showed *in vitro* antibacterial effects of phytomedicine Mathesia on *E. coli, Streptococcus pyogenes* and *Aspergillus* sp (18), and similar to those obtained by Bryskier (25).

The antibacterial activity of Mathesia on the seven bacterial isolates could be justified by some compounds present in Mathesia such as terpenes well known from their high antibacterial activity, especially the enumeration of the microbial membrane (18,26,27). This activity could also be justified by the presence of phenolic acids, tannins and flavonoids (18) which possess antibacterial properties (28,29).

Conclusion:

We showed in our study the broad-spectrum antibacterial activity of Mathesia on Gram-positive and Gram-negative bacterial pathogens isolated from chronic wound infections. This antibacterial activity could be attributed to compounds present in Mathesia such as terpenes, phenolic acids, tannins and flavonoids. The results of our study reaffirms that Mathesia could be classified as a natural broad-spectrum antibacterial plant that is useful in treating most infected wounds.

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Contributions of authors:

HNL, OK, and JPNKN conceptualized and designed the study; HNL, ZML, PNM, OLM, and CAI were responsible for data collection, analysis, and manuscript writing; JPNKN, MM, CMM, JMK, JKS, and TKM reviewed and edited the manuscript. All authors reviewed and approved the final manuscript submitted.

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No conflict of interest is declared.

References:

1. Coller, M. Wound bed management, key principals and practice: prof. Nurse; 2002; 18: 221-225.
2. Bowler, P., Duerden, B., and Armstrong, D. Wound microbiology and associated approaches to wound management: Clin. Microbiol. Rev. 2001; 14 (2): 244-269.
3. Patel, S. Investigating: Wound infection: Wound Essential, 2010; 5: 40-47.
4. Ayton, M. Wound care. Wounds that won't heal. Nurs. Times, 1985; 81: 16-19.
5. Calvin, M. Cutaneous wound repair. Wounds. 1998; 10 (1): 12-32.
6. Brook, I. Aerobic and anaerobic microbiology of necrotizing fasciitis in children. Pediatric Dermatol. 1996; 13:281-284.
7. Madsen, S. M., Westh, H., Danielson, L., and Rosadahi, V. T. Bacterial colonization and healing of venous leg ulcers. APMIS. 1996; 104: 895-899.
8. Danielson, L., Balster, E., Doring, G., Ulcer bed infection. Report of a case of enlarging venous leg ulcer colonized by *Pseudomonas aeruginosa*. APMIS. 1998; 106: 721-726.
9. Shittu, A. O., Kolawole, D. O., and Oyedepo, E. A. A study of wound infection in two health institutions in Ile-Ife Nigeria. Afri J Biomed Res. 2002; 5: 97-102.
10 Trengove, N. J., Stacey, M. C., McGechie, D. F., and Mata, S. Qualitative bacteriology and leg ulcer healing. J Wound Care. 1996; 277-280.
11 Shimekaw, M., Tigabu, A., and Tessema, B. Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors Among Patients with Wound Infections at Debre Markos Referral Hopital, Northwest, Ethiopia. Int J Lower Extermity Wounds. 2020; (00): 01-11.
12 Commons, R. J., Raby, E., Bhally, H., et al. Managing diabetic foot infections: A survey of Australasian infectious diseases clinicians. J. Foot Ankle Res. 2018; 11: 13.
13 Heravi, F. S, Zakrzewski, M., Vickery, K., Armstrong, D. G., and Hu, H. Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. J. Clin. Med. 2019; 8: 1935.
14 Godebo, G., Kibru, G., and Tassew, H. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Ann Clin Microbiol Antimicrob. 2013; 12: 1-17.
15 Mama, M., Abdissa, A., and Sewunet, T. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. Ann Clin Microbiol Antimicrob. 2014; 13: 14.
16 Ehssan H. O., Moglad, S. K.., Abdalla, B., and Hatim T. O. Ali Various Medicinal Plants: A promising treatment for multidrug-resistant bacteria isolated from wound infection. IJPSR. 2020; 11 (2): 839-843.
17 Kabedi. B. M., Kayembe, N. J. M., Kashongwe, M. Z., et al. In vitro evaluation of Mycobacterial activity of Phytomedicine Mathesia on *Mycobacterium tuberculosis*. Med Clin Rev. 2018; 4: 1-7.
18 Nkasa, L. H., Liyongo, I. C., Muzomwe, M. J. C., Masengo, I. E., Muzomwe, M. C., and Taba, K. M. Phytochemical Screening and Antibacterial Activity of Phytomedicine Mathesia; a drug use against Buruli ulcer in Republic Democratic of Congo (DRC). Euro J Pharmaceut Med Res. 2020; 7 (2):52-56.
19 Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin Pathol. 1966; 45 (4): 493-496.
20 Jehl, F., Chabaud, A., and Grillon, A. l'Antibiogramme: diamètres ou CMI? J Ant-Infect. 2015; 17 (4): 125-139.
21 Humphries, R M., Kircher, S., Ferrell, A., et al. The continued value of disk diffusion for assessing anti-microbial susceptibility in clinical laboratories: Report from the clinical and laboratory standards institute Methods Development and standardization working Group. J Clin Microbiol.2018; 56 (8): e00437-18. doi: 10.1128/JCM.00437-18.
22 Tshitenge, B. T., Ngbolua, K., Ashande, C. M., et al. Antibacterial activity of three validated Anti-sickling plant species from the great apes Pharmacopoeia in Congo Kinshasa. Int J Biomed Clin Sci. 2020; 5 (1): 33-40.
23 Berche, P., Gaillard, J. L., Simonet, M. Les bactéries des infections humaines. Editeur Flammarion Médecine-Science 1991; 660.
24 Bongo, N. G., Inkoto, C., Masengo, C., et al. Anti-sickling Antioxidant and Antibacterial Activities of *Afromomum alboviolaceum* (Ridley) K Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. F.) Skeels. Am J Lab Med. 2017; 2 (4): 52-59.
25 Bryskier, A. Fluoroquinolones (II) : Usage en therapeutique et tolerance. Encycl. Med Chi, Maladies Infectieuses. 1999; 8 (4): 11-14.
26 Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur H. D. Phytochemical Screening and Extraction a review. Int Pharmaceut Sci. 2011; 1: 98-106.
27 Ngbolua, K. N., Inkoto, C. L., Mongo, N. L., Shande, C. M. A., Masens, Y. B., and Mpiana, P. T. Etude ethnobotanique et floristique de quelques plantes médicinales commercialisée à Kinshasa, République Démocratique du Congo. Rev Mar Sci Agron Vét. 2019; 7 (1): 118-128.
28 Bongo, G. N., Ngbolua, K., Mpiana, P. T., and Tshilanda, D. D. Microscopy features, Phytochemistry and Bioactivity of Mondia Whitei L. (HOOK F.) (Apocynaceae). A mini-review. 2018; 5 (3): 34-42.
29 Inkoto, C. L., Ngbolua, K., Iteku, J. B., Tshilanda, D. D., Tshibangu, D. S., and Mpiana, P. T. Microscopic features and phytochemistry of two Congolese medicinal plants: *Afromomum alboviolaceum* (Ridley) K. Schum and *Afromomum angustifolium* (Sonn), K. Schum. (Zingiberaceae). Int J Life Sc Res Arch. 2021; 01(02): 043-052.

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Short Communication **Open Access**

Prevalence and phenotypic characteristics of *Acinetobacter baumannii* isolated from critically ill patients in two healthcare facilities in Ebonyi State, Nigeria

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Abstract:

Background: The intrinsic property of *Acinetobacter baumannii* to survive in harsh conditions on environmental surfaces and its ability to resist commonly used antibiotics in hospitals make this pathogen to be one of the most prevalent causes of hospital infections. The present study was aimed at determining the prevalence of *A. baumannii* among critically ill patients in two tertiary hospitals; Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA) and Mater Misericodiae Hospital Afikpo (MMHA) in Ebonyi State, southeast Nigeria.
Methodology: This was a hospital-based cross-sectional study of 300 consecutively selected critically ill hospitalized patients in the two hospitals over a period of 6 months, from whom a total of 300 different clinical samples were collected. The specimens were processed by standard microbiological culture methods at the Applied Microbiology Laboratory Unit of Ebonyi State University (EBSU), Abakaliki. All isolated bacteria from cultures were phenotypically screened for *A. baumannii* by conventional biochemical test scheme and antibiotic susceptibility of test (AST) of confirmed isolates was done using the Kirby–Bauer disc diffusion technique, with AST results interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline.
Results: Of the 300 critically ill patients, clinical samples of 21 (7.0%) were positive for *A. baumannii*, with 20 (10.0%) of 220 samples from AE-FUTHA and 1 (1.3%) of 80 samples from MMHA. Analysis of the different isolation sites showed that catheter urine (16.0%, 11/70) from AE-FUTHA and (2.0%, 1/50) from MMHA was the most frequent site of *A. baumannii* isolation. *A. baumannii* isolates showed high resistance rates to tetracycline (100.0%), trimethoprim-sulphamethoxazole (100.0%), ceftriaxone (81.0%) and amikacin (81.0%), while low resistance rate was demonstrated to meropenem (14.3%), imipenem (19.0%) and polymyxin B (33.3%). The multiple antibiotic resistance index (MARI) of the *A. baumannii* isolates was 12.1, with average MARI value of 0.57.
Conclusion: Early diagnosis of infection caused by *A. baumannii* and its treatment with meropenem, imipenem or polymyxin B can reduce the risks of mortality and morbidity in *A. baumannii* infection of critically ill patients.

Keywords: *Acinetobacter baumannii*, Critical illness, Prevalence, southeast Nigeria

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Prévalence et caractéristiques phénotypiques d'*Acinetobacter baumannii* isolées chez des patients gravement malades dans deux établissements de santé de l'État d'Ebonyi, Nigéria

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Résumé:

Contexte: La propriété intrinsèque d'*Acinetobacter baumannii* de survivre dans des conditions difficiles sur des surfaces environnementales et sa capacité à résister aux antibiotiques couramment utilisés dans les hôpitaux font

de cet agent pathogène l'une des causes les plus répandues d'infections hospitalières. La présente étude visait à déterminer la prévalence d'*A. baumannii* parmi les patients gravement malades dans deux hôpitaux tertiaires ; Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA) et Mater Misericodiae Hospital Afikpo (MMHA) dans l'État d'Ebonyi, au sud-est du Nigeria.

Méthodologie: Il s'agissait d'une étude transversale en milieu hospitalier portant sur 300 patients hospitalisés dans un état critique sélectionnés consécutivement dans les deux hôpitaux sur une période de 6 mois, auprès desquels un total de 300 échantillons cliniques différents ont été collectés. Les échantillons ont été traités par des méthodes de culture microbiologique standard au laboratoire de microbiologie appliquée de l'Université d'État d'Ebonyi (EBSU), à Abakaliki. Toutes les bactéries isolées des cultures ont été analysées phénotypiquement pour A. baumannii par un schéma de tests biochimiques conventionnels et le test de sensibilité aux antibiotiques (AST) des isolats confirmés a été effectué à l'aide de la technique de diffusion sur disque de Kirby-Bauer, les résultats de l'AST étant interprétés conformément aux normes cliniques et de laboratoire. Lignes directrices de l'Institut (CLSI).

Résultats: Sur les 300 patients gravement malades, 21 échantillons cliniques (7,0%) étaient positifs pour *A. baumannii*, dont 20 (10,0%) sur 220 échantillons provenant d'AE-FUTHA et 1 (1,3%) sur 80 échantillons provenant de MMHA. L'analyse des différents sites d'isolement a montré que l'urine de cathéter (16,0 %, 11/70) de l'AE-FUTHA et (2,0%, 1/50) du MMHA était le site le plus fréquent d'isolement d'*A. baumannii*. Les isolats d'*A. baumannii* ont montré des taux de résistance élevés à la tétracycline (100,0%), au triméthoprime-sulfaméthoxazole (100,0%), à la ceftriaxone (81,0%) et à l'amikacine (81,0%), tandis qu'un faible taux de résistance a été démontré au méropénème (14,3%), à l'imipénème (19,0%) et polymyxine B (33,3%). L'indice de résistance multiple aux antibiotiques (MARI) des isolats d'*A. baumannii* était de 12,1, avec une valeur moyenne du MARI de 0,57.

Conclusion: Le diagnostic précoce de l'infection causée par *A. baumannii* et son traitement par méropénème, imipénème ou polymyxine B peuvent réduire les risques de mortalité et de morbidité liés à l'infection à *A. baumannii* chez les patients gravement malades.

Mots clés: *Acinetobacter baumannii*, Maladie grave, Prévalence, sud-est du Nigeria

Introduction:

The genus *Acinetobacter* is a group of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria (1). This group of organisms can survive for prolonged periods of time in the environment and on the hands of healthcare workers (2). *Acinetobacter* is a clinically important pathogen with widespread resistance to various antibiotics. The bacterium is a key cause of infection among debilitated patients in the hospital (1).

Initially, the *Acinetobacter calcoaceticus-baumannii* (ACB) complex comprised four species; *Acinetobacter calcoaceticus* (genomic species 1)*, Acinetobacter baumannii* (genomic species 2), *Acinetobacter pittii* (previously named genomic species 3) and *Acinetobacter nosocomialis* (previously named genomic species 13 TU). Of these, *A. baumannii* is the most important clinically relevant species, responsible for 80% of *Acinetobacter* infections (3,4).

Acinetobacter baumannii has been reported as a notorious opportunistic pathogen, affecting debilitated patients especially at the intensive care units (ICU) and others with underlying illnesses, and the bacterium has consistently jeopardized many antibiotics (5). The clinical significance of *A. baumannii* as a hospital-acquired pathogen is undoubtedly related to its resistance to commonly used antibiotics and virulence potentials (4).

Although *A. baumannii* was initially considered as a low virulence pathogen, recent studies have shown *A. baumannii* as one of the most significant clinical pathogens associated with hospital-acquired infections (5-8). Pneumonia has been the major disease manifestation of nosocomial infections caused by this pathogen, resulting in significantly high mortality rate of patients. It is also responsible for wide range of other infections including septicemia, meningitis, urinary tract infection, endocarditis, and more recently, severe and deadly cases of necrotizing fasciitis (9).

Acinetobacter baumannii is recognized as one of the six ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) by the Infectious Diseases Society of America (IDSA). It has subsequently developed into a pan-drug resistant (PDR) pathogen and received rapid recognition as one of the most important bacterial pathogens causing healthcare-associated infections (10,11). This high MDR attributes and persistence make *A. baumannii* a serious threat to hospitalized patients.

The hallmark of extreme or extended drug resistance (XDR) phenotype is carbapenem resistance (CR), and carbapenem resistant *A. baumannii* (CRAB) constitute the major strains in many hospitals today (10,11), and are now reported as important cause of different types of infections including endocarditis, skin and soft tissue infections, meningitis, septicemia, respiratory and urinary tract infections. Carbapenem-resistant strains are often resistant to all other routinely available antibiotics except polymyxins (colistin), tigecycline, and sometimes aminoglycosides (10,11). Treatment of CRAB infections therefore involves the use of combinations of last resort antibiotics such as colistin (12).

High costs of treatment of CRAB infections, treatment failure, and high mortality have been reported in various health facilities

globally. In view of the paucity of information on infections caused by *A. baumannii* in Alex Ekwueme-Federal University Teaching Hospital Abakaliki (AE-FUTHA) and the Mater Misericodiae Hospital Afikpo (MMHA) Ebonyi State, Nigeria, this study was aimed at determining the prevalence and antibiotic susceptibility of *A. baumannii* isolates among critically ill patients hospitalized in the various wards and intensive care units of the two hospitals.

Materials and method:

Study area:

The study was conducted at the Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA), and Mater Misericodiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria. Ebonyi State is located in southeastern Nigeria within longitude 7.30' and 8.30'E and latitude 5.40' and 6.45'N. The State was created on October 1, 1996 from the former Abia and Enugu States, with Abakaliki as its capital. It is bounded to the north by Benue State, to the west by Enugu State, to the east by Cross River State and to the south by Abia State.

There are thirteen Local Government Areas (LGAs) in the State namely; Abakaliki, Ebonyi, Ishielu, Ohaukwu, Izzi, Ikwo, Ezza North, Ezza South, Afikpo North, Afikpo South, Ivo, Ohaozara and Onicha LGAs. There are many government-owned and some private health clinics are obtained from the study area.

Study design and period:

This study was hospital-based cross-sectional design involving 300 critically ill patients on admission in medical, surgical, and orthopedic wards, and the intensive care unit (ICU) of AE-FUTHA and MMHA. The study was conducted over a period of 6 months (September 1, 2022 – March 1, 2023)

Ethical clearance:

Ethical clearance was obtained from Research and Ethical Committee (REC) of AE-FUTHA (AE-FUTHA/REC/VOL3/2022/129) and written permission was obtained from the management of MMHA. Participation in the study was highly voluntary and participants who consented were at will to withdraw from participation at any point they felt uninterested. Informed consent was obtained from the participants or their spouse, parents or caregiver.

Sample size and participant selection:

The sample size was estimated using the Leslie Kish formula (13), which gave the calculated minimum number of participants as 300. The participants were critically ill patients who had been hospitalized for at least 14 days in male and female medical wards, male and female surgical wards, male and female orthopedic wards, burns, and intensive care units (ICU) of the hospital. The participants were recruited by consecutive sampling over the period of study.

Data and sample collection:

Relevant socio-demographic data and other information were obtained from the participants care giver or spouse. Demographic data collected included age, gender, marital status, duration of hospital stay and ward of admission. Different clinical samples as appropriate to each patient condition were aseptically collected using sterile urine container or by sterile swab sticks, and these included catheter urine, wound drain, and swabs of wound, skin, nose, and mouth. All samples were transferred to the Applied Microbiology Laboratory Unit of the Ebonyi State University (EBSU) for microbiological analysis.

Culture and isolation of *Acinetobacter:*

Wound swab was first inoculated onto Nutrient broth (Merck, Germany) and incubated aerobically for 24 hours followed by subculture on MacConkey agar (Merck, Germany) and further incubation for 24 hours at 37°C. Urine samples were directly inoculated onto MacConkey agar. *Acinetobacter* grew on MacConkey agar as non-lactose fermenter (colorless or slightly beige), and was presumptively identified as Gram-negative bacilli or coccobacilli (on Gram stain), oxidase negative, catalase positive and non-motile by hanging drop technique.

Acinetobacter isolate was phenotypically confirmed as *A. baumannii* by growth at 37°C and 42°C and by other biochemical tests (14). *Acinetobacter baumannii* ATCC1605 was used as a positive control for each test protocol.

Antimicrobial sensitivity testing:

Antibiotic susceptibility test on each isolate was done by the disk diffusion method as previously described (15). The colony suspension from each overnight culture was prepared using nutrient broth and compared with the turbidity of 0.5 McFarland standards. With the aid of a sterile swab stick, Mueller-Hinton agar plates were inoculated with suspension of the organism and allowed for 30 mins for prediffusion. Antibiotic impregnated discs (Oxoid, UK) including imipenem (10µg), tetracycline (10µg), meropenem (10µg), sulfamethoxazole-trimethoprim (25µg), amikacin (30µg), ciprofloxacin (5µg), polymyxin B (300 unit), ceftriaxone (30µg), doxycycline (10µg) and gentamicin (10µg), were placed on the surface of the media and incubated at 37° C for 24 hrs. The inhibition zone diameters were measured using a meter rule and the isolates were class-

ified as susceptible or resistant according to the Clinical and Laboratory Standards Institute guideline (16).

Multi-drug resistance (MDR) *A. baumannii* was taken as simultaneously resistance to three or more classes of antibiotics such as extended-spectrum cephalosporins (ceftriaxone, cefotaxime, ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin), aminoglycosides (gentamicin, amikacin), β-lactamase and β-lactamase inhibitors (ampicillin-sulbactam) and carbapenems (imipenem, meropenem, ertapenem).

Multiple antibiotic resistance index (MARI) was calculated and interpreted according to the method described by Ayandele et al., (17) using the formula; MARI=a/b, where 'a' is the number of antibiotics to which a particular isolate is resistant to and, 'b' is the total number of antibiotics tested against the isolate.

Results:

A total of 300 critically ill patients (220 from AE-FUTHA and 80 from MMHA) were recruited and clinical samples collected from each of them from the different wards of the two hospitals. Of these, 153 (51.0%) were males while 147 (49.0%) were females, with 113 males and 107 females from AE-FUTHA, 40 males and 40 females from MMHA. The age of the patients ranged from 18-69 years, with majority in age group 30-39 years (n=124) and 40-49 years (n=113), while the least is in the age group 60-69 years (n=1) (Table 1).

Of the 300 patients, 21 (7.0%) samples were positive for *A. baumannii* with 20 of the 220 participants (10.0%) from AE-FUTHA and 1 of the 80 participants (1.3%) from the MMHA being positive (Table 1). The most frequent *A. baumannii* infection occurred in the

Table 1: Frequency and demographic characteristics of critically ill patients with *A. baumannii* infections at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Characteristics	**AE-FETHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
All patients	220	20 (10.0)	80	1 (1.3)	300	21 (7.0)
Gender						
Male	113	12 (10.6)	40	1 (2.5)	153	13 (8.5)
Female	107	8 (7.5)	40	0	147	8 (5.4)
Age group (years)						
18-29	31	3 (9.7)	22	0	53	3 (5.7)
30-39	88	4 (4.5)	36	1 (2.8)	124	5 (4.0)
40-49	94	11 (11.7)	19	0	113	11 (9.7)
50-59	6	2 (33.3)	3	0	9	2 (22.2)
60-69	1	0	0	0	1	0

Table 2: Frequency of critically ill patients with *Acinetobacter baumannii* infection by the hospital wards/units at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Ward of admission	**AE-FUTHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
Medical ward	89	15 (16.9)	29	0	118	15 (12.7)
Orthopaedic ward	65	1 (1.5)	36	1 (2.8)	101	2 (1.9)
Intensive care unit	5	1 (20.0)	0	0	5	1 (20.0)
Surgical ward	61	3 (4.9)	15	0	76	3 (3.9)
Total	**220**	**20 (10.0)**	**80**	**1 (1.3)**	**300**	**21 (7.0)**

age group 50-59 years (22.2%, 2/9) followed by age group 40-49 years (9.7%, 11 of 113), while no infection was reported in the age group 60-69 years (Table 1).

Table 2 shows the different wards of admission and the frequency of isolation of *A. baumannii* from the clinical samples of patients. Most samples were collected from patients in medical (n=118) and orthopaedic (n= 101) wards followed by surgical ward (n=76) and very few from the intensive care unit (n=5). *A. baumannii* were isolated from all the wards and ICU of AE-FUTHA with most frequent being from the ICU (20.0%, 1/5) followed by medical (16.9%, 15/89), surgical (4.9%, 3/61) and orthopaedic (1.5%, 1/65) wards. However, the only *A. baumannii* isolated from MMHA was from orthopaedic ward.

As shown in Table 3, the most frequent source of clinical samples from the patients were catheter urine (n=120) and wound ulcer (n=82). *A. baumannii* were isolated most frequently from catheter urine (10.0%, 12 of 120), followed by wound drain (7.7%, 2/26), wound ulcer (7.3%, 6/82) and skin swab (6.7%, 1/15). *A. baumannii* were isolated from all the samples in AE-FTHA except from skin and nose swabs while the only *A. baumannii* isolated from MMHA was from catheter urine.

The antimicrobial susceptibility test of the 21 *A. baumannii* isolates to 10 commonly used antibiotics showed the highest levels of resistance to tetracycline (100.0%), trimethoprim-sulphamethoxazole (100.0%), followed by amikacin (80.9%), ceftriaxone (80.9%), gentamicin (57.0%), doxycycline (47.6%), and ciprofloxacin (42.8%), while resistance to meropenem, imipenem and polymyxin B were low 14.2%, 19.0% and 33.3%) respectively (Table 4).

The MARI value of the total *A. baumannii* isolates was 12.1 while the average MARI value was 0.57. Bacteria having MARI (>0.2) originate from a high-risk source of contamination where antibiotics are widely used. MARI value of ≤0.2 indicates strain originated from sources where antibiotics are seldom or never used.

Table 3: Frequency of critically ill patients with *A. baumannii* infections with respect to clinical samples at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Sample source	**AE-FUTHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
Catheter urine	70	11 (15.7)	50	1 (2.0)	120	12 (10.0)
Wound ulcer	67	6 (9.0)	15	0	82	6 (7.3)
Wound drain	24	2 (8.3)	5	0	26	2 (7.7)
Skin swab	13	1 (7.7)	2	0	15	1 (6.7)
Nose swab	20	0	5	0	25	0
Mouth swab	26	0	3	0	29	0
Total	**220**	**20 (10.0)**	**80**	**1 (1.0)**	**300**	**21 (7.0)**

Table 4: Antibiotics susceptibility of *A. baumannii* isolates from clinical samples of critically ill patients in Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordia Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Antibiotic	**Disc strength**	**No of isolates resistant (%)**	**No of isolates sensitive (%)**
Ciprofloxacin	5 µg	9 (42.9)	12 (57.1)
Tetracycline	5 µg	21 (100.0)	0
Trimethoprim-sulphamethoxazole	25 µg	21 (100.0)	0
Imipenem	10 µg	4 (19.0)	17 (81.0)
Gentamicin	10 µg	12 (57.1)	9 (42.9)
Amikacin	30 µg	17 (81.0)	4 (19.0)
Meropenem	10 µg	3 (14.3)	18 (85.7)
Doxycycline	10 µg	10 (47.6)	11 (52.4)
Polymyxin B	300 unit	7 (33.3)	14 (66.7)
Ceftriaxone	30 µg	17 (81.0)	4 (19.0)

Discussion:

Infection due to *Acinetobacter* species is a major public health challenge within the health care facilities and the community in general due to its multidrug resistance even to the most potent drugs such as carbapenems. Members of the genus *Acinetobacter* have not only shown increasing resistance to β-lactams but also to other classes of antibiotics such as aminoglycoside antibiotics and also thought to be a reservoir of antibiotic resistant genes in hospital environment (18,19). The prevalence of *A. baumannii* infection/colonization in the study is 7.0% (21/300), with the most frequent site of infection being the urinary tract and surface wound. This agrees with studies in Nigeria and elsewhere where urine and wound exudates/pus have been reported to be the most frequent clinical specimens sent for isolation of *A. baumannii* pathogen. However, Mohammad (20) and Neetu et al., (21) in Bangalore reported recovery of *A. baumannii* most frequently from blood samples

The number of critically ill patients in the intensive care unit (ICU) of the AE-FUTHA was relatively small, while there was no patient in ICU at MMHA during the entire period of the study. In spite of this, at AE-FUTHA, *A. baumannii* was more frequently isolated (20%, 1/5) from the ICU when compared to the medical (16.9%, 15/89) and surgical (4.9%, 3 of 61) wards. This is line with the findings of some studies conducted in different parts of Nigeria and in other parts of the world. In Nigeria, Nwadike et al., (18) reported ICU as the major point of isolation of *A. baumannii,* while a prevalence of 18.4 % was reported by Natalia et al., (8) at the surgical ICU of Maryland Medical Center, USA, and Neetu et al., (21) also reported high isolation rate from ICU of the hospital in Padmashree Bangalore. In contrast, Ikechukwu et al., (5) in Nigeria reported highest isolation rate from medical ward.

The antibiotic susceptibility test result showed that *A. baumannii* isolates in our study were mostly resistant to at least 3 classes of antibiotics, making them to be multidrug-resistant (MDR) isolates. The highest of resistance of the isolates was to tetracycline and trimethoprim-sulfamethoxazole while the least resistance was to meropenem, imipenem and polymyxin, which is in agreement with the finding of Ikechukwu et al., (5) who reported high *in vitro* efficacy of meropenem and imipenem against *A. baumannii*. Direkel et al., (22) and Eghbalimoghadam et al., (23) also reported similar low resistance rate to imipenem in Turkey and Iran respectively. Our findings and those of others suggest that meropenem and imipenem still remain the most potent antibiotics against *A. baumannii*.

The resistance to gentamicin was relatively high (57.1%), and quite high to ceftriaxone (80.0%) and tetracycline (100%) in the current study. A similar study by Muhammed (20) reported high resistance of *A. baumannii* to gentamicin (94.3%) and tetracycline (95%) but relatively low resistance rate to ceftriaxone (35.0%). Contrarily, the study by Al-Agamy et al., (24) reported that *A. baumannii* isolates were highly resistant to ciprofloxacin (80.0%) and imipenem (70.0%). The difference in resistance rates reported may reflect the degree of antibiotic exposure and use/ misuse in different settings.

The result of MARI of the isolates in our study agrees with that of Onuoha et al., (15) who reported average MARI of their isolates to be higher than 0.20. Bacteria having MARI > 0.2 tends to originate from a setting with high levels of antibiotics exposure, while those with MARI ≤ 0.2 tend to originate from setting with low antibiotic exposure.

Conclusion:

The prevalence of *A. baumannii* infection/colonization of critically ill patients in this study is 7.0%, which emphasizes the clinical importance of this pathogen in this group of patients. The most frequent sites of infection/ colonization are the urinary tracts and surface wounds, and the organism exhibited multidrug resistance pattern. However, meropenem and imipenem showed high *in vitro* activity and are recommended for treatment of infections caused by *A. baumannii* in critically ill patients.

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Contributions of authors:

OO conceived and designed the study, collected and analyze the samples; OSC wrote and reviewed the manuscript, DIE, OCN, EBO and OO carried out the literature search and critical review of the manuscript. All authors read and approved the final manuscript.

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References:

1. Bitrian, M., González, H., Paris, G., Hellingwerf, J., and Nudel, B. Blue-light-dependent

inhibition of twitching motility in *Acinetobacter baylyi* ADP1: additive involvement of three BLUF-domain-containing proteins. Microbiol. 2013; 159 (9):1828–1841. doi: 10.1099/mic.0.069153-0
2. Zeleke, A., Eyasu, T., Elias, S., Semira, E., Dawit, A., and Estifanos, T. Multidrug resistance pattern of *Acinetobacter* species isolated from clinical specimens. Ethiop Publ Health Inst 2021; 16 (4): 2508. doi:10.1371/journal.pone.0250896
3. Chusri, S., Chongsuvivatwong, V., Rivera J. I., et al. Clinical outcomes of hospital-acquired infection with *Acinetobacter nosocomialis* and *Acinetobacter pittii*. Antimicrob Agents Chemother. 2014; 58(7): 4172-4179 doi: 10.1128/AAC.02992-14
4. Nemec, A., Krizova, L., and Maixnerova M. *Acinetobacter seifertii* sp. nov., a member of the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex isolated from human clinical specimens. Int J Syst Evol Microbiol. 2015; 65 (3): 934–942 https://doi.org/10.1371/journal.pone.0250896
5. Ikechukwu, E., Ifeanyichukwu, I., Modesta, E. et al. Antimicrobial Susceptibility Pattern and Molecular Identification of *Acinetobacter baumannii* in Alex Ekwueme-Federal University Teaching Hospital Abakaliki, Nigeria. J Pharmaceut Res Int 2021; 33 (44B): 409 - 419. doi:10.9734/jpri/2021/v33i44B32691
6. Chao, L., Yaowen, C., Ying, X., et al. Distribution of virulence-associated genes and antimicrobial susceptibility in clinical *Acinetobacter baumannii* isolates. J Antimicrob Chemother. 2018; 24; 9 (31): 21663 - 21673. doi: 10.18632/oncotarget.24651
7. Lin, M. F., and Lan, C. Y. Antimicrobial resistance in *Acinetobacter baumannii*: From bench to bedside. World J Clin Cases**.** 2014; 2: 787–814. doi: 10.12998/wjcc.v2.i12.787
8. Natalia, B., Anthony, D. H., Clark, C., et al. Risk factors and outcomes associated with multidrug-resistant *Acinetobacter baumannii* upon intensive care unit admission. Antimicrob Agents Chemother 2018; 62(1):1-17. doi: 10.1128/AAC.01631-17
9. Gaddy, J. A., Arivett, B. A., McConnell, M. J., Lopez-Rojas, R., Pachon, J., and Actis, L. A. Role of Acinetobactin-mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606T with human lung epithelial cells, Galleria mellonella caterpillars, and mice. Infect Immunol. 2012; 80: 1015-1024. doi: 10.1128/AAC.01631-17
10. Falagas, M. E., and Bliziotis, I. A. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era. Int J Antimicrob Agent. 2007; 29: 630–660. doi:10.1016/j.ijantimicag.2006.12.012
11. Falagas, M. E., and Rafailidis P. I. Attributable mortality of *Acinetobacter baumannii*: no longer a controversial issue. Critical Care. 2007; 11: 134. doi: 10.1186/cc5911
12. Yeom, J., Shin, J., Yang, J., Kim, J., Hwang, G. and Bundy, J. G. NMR-Based Metabolite Profiling of Planktonic and Biofilm Cells in *Acinetobacter baumannii*. PLoS One. 2013; 8 (3): 1656 - 1659. https://doi.org/10.1371/journal.pone.0057730
13. Kish, L. Survey Sampling. New York: John Wiley and Sons, Inc. 1965.
14. Cheesbrough, M. Bacteriological testing of water: In District Laboratory Practice in Tropical Countries. 2000. Part 2: 149-154.
15. Onuoha, S. C., Okafor, C. O., Aduo, B. C., and Nwaka. F. C. Distribution of Antibiotic Resistant Bacteria from Abattoir Wastes and its Receiving Waters at Nkwo-ezzamgbo, Ebonyi State, Nigeria. World J Med Sci. 2016; 13 (4): 242-250. doi 10.5829/idosi.wjms.2016.242.250
16. Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing M02-A12, M07-A10, and M11-A8. 27th Edition; 2017: 282
17. Ayandele, A., Oladipo, E. K., Oyebisi, O., and Kaka, M. O. Prevalence of Multi-Antibiotic Resistance *E. coli* and *Klebsiella* species obtained from a Tertiary Medical Institution in Oyo State, Nigeria. Qatar Med J. 2020; 1**:** 9-13. doi: 10.5339/qmj.2020.9
18. Nwadike, V. U, Ojide, C. K., and Kalu, E. I. Multidrug Resistant *Acinetobacter* infection and their Antimicrobial Susceptibility Pattern in a Nigerian Tertiary hospital ICU. Afr J Infect Dis. 2014; 8 (1): 14 – 18 http://dx.doi.org/10.4314/ajid.v8i1.4.
19. Boon, H. K., Yasmin, A. H., Mohd, M. Y. and Kwai, L. T. Antimicrobial Susceptibility Profiling and Genomic Diversity of Multidrug-Resistant *Acinetobacter baumannii* Isolates from a Teaching Hospital in Malaysia. Jpn J Infect Dis. 2011; 64: 337-340. doi: 10.7883/yoken.64.337
20. Muhammed, D. Virulence Factors Profile and Antimicrobial Resistance of *Acinetobacter baumannii* Strains Isolated from Various Infections Recovered from Immuno suppressive Patients. Biomed Pharmacol J. 2016; 9 (3):1057-1062. https://dx.doi.org/10.13005/bpj/1048
21. Neetu, G., Nageswari, G.,, Savita J., and Ravindra, M. Isolation and identification of *Acinetobacter* species with special reference to antibiotic resistance. J Nat Sci Biomed. 2015; 6 (1): 159–162. doi:10.4103/0976-9668.149116
22. Direkel, S., Copur, C. A., Karagoz, A., et al. Antimicrobial susceptibility and molecular characterization of multidrug-resistant *Acinetobacter baumannii* isolated in a University Hospital. Microbiol Bulletin. 2016; 50:522–534. doi; 10.5578/mb.34158
23. Eghbalimoghadam, M., Farahani, A., Akbar, F. N., Mohajeri, P. Frequency of Class 1 Integron and Genetic Diversity of *Acinetobacter baumannii* Isolated from Medical Centers in Kermanshah. J Nat Sci Biol Med. 2017; 8: 193–198. doi: 10.4103/0976-9668.210007
24. Al-Agamy, M. H., Khalaf, N. G., Tawfick, M. M., Shibl, A. M., and El Kholy, A. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt. Int J Infect Dis. 2014; 22:49–54 doi; 10.1016/j.ijid.2013.12.004

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