GET Journal of Biosecurity and One Health

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Message from the Editor-in-Chief

Prof. Akin Abayomi
Editor-in-Chief

Dear Esteemed Readers,

It is with great pleasure that I welcome you to Volume 3, Issue 1 of the GET Journal of Biosecurity and One Health. This edition showcases a collection of insightful research articles addressing critical issues at the intersection of biosecurity, antimicrobial resistance, and public health. The studies featured delve into topics such as the spatial distribution of malaria parasites and their vectors, antimicrobial resistance patterns across states in Nigeria, and the molecular detection of extended-spectrum beta-lactamases in clinical isolates. These articles underscore the persistent challenges posed by infectious diseases and antimicrobial resistance while offering innovative perspectives on their mitigation.

One notable study explores the potential of natural plant extracts, such as those from Spondias mombin (Linn), as alternatives for combating multiple antibiotic-resistant bacteria. Such research exemplifies the diverse approaches needed to address the global health threats posed by resistant pathogens. By presenting these and other findings, this issue contributes to the broader understanding of strategies necessary to safeguard public health in Africa and beyond.

The GET Journal of Biosecurity and One Health continues to provide a vital platform for the dissemination of high-quality, transdisciplinary research. Through our commitment to publishing diverse article types, including original research, reviews, short communications, and case studies, we aim to foster collaboration among researchers, practitioners, and policymakers globally.

I extend my heartfelt gratitude to our authors, reviewers, and editorial team, whose dedication and expertise have made this publication possible. Their efforts ensure the journal remains a leading resource for advancing knowledge in biosecurity and One Health. As always, we welcome submissions for upcoming issues and encourage researchers to share their work to further enrich the scholarly dialogue on these vital topics. Please visit www.getjournal.org for detailed submission guidelines. Importantly, we remain committed to open-access publishing, ensuring that valuable research is freely accessible to all without financial barriers.

Thank you for your continued support of the GET Journal of Biosecurity and One Health. Together, let us continue to drive impactful research, promote collaboration, and address the challenges that threaten global health security.

Warm regards,
Prof. Akin Abayomi
Editor-in-Chief
GET Journal of Biosecurity and One Health

ABOUT GET JOURNAL

GET Journal of Biosecurity and One Health is an international scholarly peer reviewed Open Access journal that aims to promote research in all the related fields of Biosecurity and One Health. The United Nations Food and Agriculture Organisation defines biosecurity in the context of a strategic and integrated approach that encompasses the policy, regulatory frameworks, instruments, and activities for analysing and managing relevant risks to human, animal and plant health, and associated risks to the environment. Biosecurity covers food safety, zoonoses, the introduction of animal and plant diseases and pests, the introduction and release of living modified organisms (LMOs) and their products (genetically modified organisms or GMOs), and the introduction and management of invasive alien species. The GET Journal of Biosecurity and One Health is devoted exclusively to the publication of high-quality research papers that covers multidisciplinary fields of Biosecurity and One Health. The journal aims to publish high quality varied article types such as Research, Reviews, Short Communications, Case Reports, Perspectives (Editorials), Clinical Images.

AIMS AND SCOPE

GET Journal of Biosecurity and One Health is an international scholarly peer reviewed Open Access journal aimed at promoting research and publishing high quality articles in all the related fields of Biosecurity and One Health.

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EDITORIAL BOARD

Prof. Akin Abayomi is the Honourable Commissioner for Health, Lagos State, an experienced and versatile Medical Doctor who has served as a lecturer and practitioner in Africa as well as the West Indies and has written numerous research publications on Cancer, Diabetes and Sickle Cell Anaemia. He obtained an MBBS degree from the University of London, United Kingdom and a Master of Philosophy (M.Phil) in Ecology and Environmental Health Management from the University of Pretoria, South Africa. He was a Consultant Haematologist and Lecturer at the University of Zimbabwe Medical School and Harare Group of Teaching Hospitals, Zimbabwe, between 1994 and 1998. He was also Chief Physician at the Princess Marina Hospital, Gabarone, Botswana in 1998.

He is a Fellow of the Royal College of Physicians of Edinburgh (2010) and the Royal College of Pathologists of the United Kingdom (2013), He was the Consultant Haematologist, Faculty of Medicine & Research, Queen Elizabeth Hospital, University of West Indies, Bridgetown, Barbados from 1998-2006. He was a Bone Marrow Transplant Research Fellow at the University of Stellenbosch as well as a Consultant Clinical Haematologist, Constantiaberg Bone Marrow Transplant Unit, Tygerberg Academic Hospital, Cape Town, South Africa. He was Head of Division, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa. He has held various positions in the field of medicine including Consultant, Lagos State Biosecurity and Genomic Project, Lead Consultant to the West African Health Authority (WAHO), ECOWAS and President, Federation of South African Society of Pathology, Nigerian Institute of Medical Researcher, (NIMR) among others.

Prof. Oluwafemi Sunday Obayori is a Professor of Environmental Microbiology with a specialization in biodegradation of petroleum hydrocarbons and bioremediation. He lectures at the Department of Microbiology, Lagos State University. He has over fortyfive publications in reputable scientific journals. He was at various times Head of Department of Microbiology and Dean of Students' Affairs, a member of the Nigeria Society for Microbiology (NSM), Society for Applied Microbiology (SFAM), and the American Society for Microbiology (ASM). His current research interests include Metagenomic insight into the bacterial resources of Lagos lagoon waters, Heavy metals, and antibiotic resistomes of pristine and polluted ecosystems. Asides from academics, Oluwafemi Obayori is a political activist with a passion for literary interrogation and expression of social reality. Which is showcased in his organizational experience and body of intellectual materials to his credit in this domain.

Prof. Akin Osibogun is an experienced professor with a demonstrated history of working in the medical practice industry. He is skilled in Epidemiology, Management, Global Health, Healthcare Management, and Healthcare. He is a strong education professional with a FMCPH (National Postgraduate Medical College of Nigeria), FWACP (West African College of Physicians) focused on Health/Health Care Administration/Management, Health Care Financing from College of Medicine, University of Lagos; Columbia University, New York; University of Zagreb, Croatia.

Prof. Charles Shey Wiysonge is the director of Cochrane South Africa at the South African Medical Research Council; an Honorary Professor of Epidemiology and Biostatistics at the University of Cape Town (UCT); and an Extraordinary Professor of Global Health at Stellenbosch University, South Africa. His previous appointments include Deputy Director of the Centre for Evidence-based Health Care and Professor of Community Health at Stellenbosch University; Chief Research Officer at UCT, South Africa; Chief Research Officer at UNAIDS, Geneva, Switzerland; Deputy Permanent Secretary in the Central Technical Group of the Expanded Programme on Immunisation, Cameroon;

He is a member of various advisory committees in the fields of research, vaccination, and evidence-based policy in Africa and globally. Professor Wiysonge obtained an MD from the University of Yaoundé I Cameroon in 1995, an MPhil from the University of Cambridge UK in 2000, and a PhD from UCT in 2012.

Prof. Angela Chukwu has over fifteen years of teaching and research in Statistics with applications in the life sciences and Public Health. She is a proficient in classical Statistical methodologies including experience in the analysis of experimental data using parametric and nonparametric methods, sampling and sample size estimation, mathematical demography, survival analysis and probability. She is committed to mentoring and facilitating international partnerships on research for national development.

Prof. Sunday Omilabu is an internationally renowned virologist with over 30 years of experience in teaching and consultancy. He is an experienced professor with a demonstrated history of working in the medical practice industry. He is currently a Director at the Centre for Human and Zoonotic Virology (CHAZVY), College of Medicine University of Lagos Lagos University Teaching Hospital (LUTH).

Prof. Sahr Gevao attended the College of Medicine, University of Lagos from 1977 -1982, graduating with a Medical Degree. He commenced residency training in Laboratory Medicine at the University College Hospital, Ibadan Nigeria specializing in Hematology and Blood Transfusion and was certified by the West African College of Physicians in 1988. His next appointments from 1989 -1992, as a research fellow, were at the Medical Research Council Laboratories, Fajara. Banjul, the Gambia, and Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom, where he was involved in varied research projects on HIV, Polio, and Sickle cell Disease. Gevao commenced an academic and professional career at the College of Medicine and Allied Health Sciences, University of Sierra Leone and Ministry of Health and Sanitation. He was Deputy Vice Chancellor and Head of the College from 2005- 2009. He served as National Manager Laboratory Services from 2009- 2013 and have extensive experience in Medical Education and Management.

Dr. Lateef Adeleke is budding scholar with bias in Law and Development in Africa. He is a Senior Lecturer in the College of Law, Crescent University, Abeokuta Ogun State Nigeria. He is currently the head, Department of Commercial and Property Law of the same College. He holds a bachelor of Law degree from Obafemi Awolowo University, Ile Ife. He has a Master's degree in African Law from the University of Ibadan, Master's degree in Common Law from the University of Ilorin and a PhD from the University of Ibadan.

Prof. Abiodun A. Denloye is a professor in the Department of Zoology and Environmental Biology at Lagos State University, Lagos, Nigeria. He is specialized in Medical and Applied Entomology with strong passion for Biosafety and Biosecurity Risk (Biorisk) Management. His pioneering efforts contributed to the formation of the Nigeria Biological Safety Association (NiBSA) in 2010. He was the pioneer Secretary of NiBSA, former Vice President and now the President. He is a well grounded Biosafety and Biosecurity expert as an International Foundation for Biosafety Associations (IFBA) Certified Biorisk Management Professional, IFBA Certified Biosecurity Professional, and Certified Biorisk Management trainer with access to the Global Biorisk Management Curriculum (GBRMC) Library. Also, he is a certified Trainer and Shipper of Biological Samples, he is well versed in deploying the science and skills underpinning decision-making in respect of the biosafety of Genetically Modified Organisms (GMOs), having trained at different times at the International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy. He creates time to engage in birding, and enjoys reading writing, and travelling as his hobbies. His forte is service, creating platforms for people to express themselves and bringing up opportunities in place of despair. He is a Fellow of the Entomological Society of Nigeria (FESN), Fellow of the Nigerian Biological Safety Association (FNiBSA) and Fellow of the Society for Educational Administrators of Nigeria (FSEAN).

Dr. Kirk Douglas is a professional senior scientist recognized both regionally and internationally for impactful scientific research in the fields of microbiology, infectious diseases, biosecurity, virology and zoonoses. He has earned a Bachelor of Science (B.Sc.) degree in Microbiology (2001), a Master of Philosophy (M.Phil.) degree in Microbiology (2007) and a Doctor of Philosophy (Ph.D.) degree in Medical Microbiology (2020) from the University of the West Indies, Cave Hill, Barbados. In addition, he holds a Master of Business Administration (MBA) degree with Merit Honours (2019) from Warwick Business School (WBS), University of Warwick, United Kingdom. Dr. Douglas commenced his career as a summer student in the Virology Department at the Hospital for Sick Kids, Toronto, Canada (2001), then upon returning home to Barbados, he worked as a Veterinary Laboratory Technician at Veterinary Services Laboratory, Ministry of Agriculture, Barbados in 2001, before moving on to an international medical device manufacturer in Barbados from 2002 until 2019. In addition, he has led several initiatives to minimize product scrap and poor quality in intraocular (IOL) manufacturing processes resulting in significant corporate savings and increased profitability. His research in the fields of infectious diseases, biosecurity and virology started as an undergraduate at UWI Cave Hill involving a summer field research project on wild rats with Professor Paul Levett, which led to his first publication as a co-author, the first report of serological evidence of hantavirus infections amongst humans and rodents in both Barbados and the Caribbean (2002). He has authored multiple peer-reviewed scientific papers in the fields of microbiology, virology, biosecurity, infectious diseases and zoonoses which have received almost 100 citations.

Dr Sam Ujewe is an expert, scholar and researcher in Bioethics, Applied Ethics and Global Health Policy with specializations in: global health inequities & social justice, ethics & health policy, moral philosophy, health research ethics, health ethics, mental health ethics, international & cross-cultural bioethics, ethics of infectious diseases, public health ethics, and healthcare decision-making. He possesses a proven ability to develop research, secure funding and manage research projects and awards; and address practical health ethics and policy issues in the light of local and international ethics guidelines and regulations. His research outlook focuses on the intersection of health ethics and public policy, aiming to establish ethical reforms in local and international policies, regulations and guidelines with real-world impact, and benefiting historically disadvantaged populations and groups.

Prof. Dorcas Yole holds a PhD in Biology from the University of York, United Kingdom. Her field of specialization is Immunology and Parasitology. She is a Professor at the Technical University of Kenya (TUK). Currently she is the Director of School of Biological and Life Sciences. Previously she was the Director, Campus Outreach Programmes. Prof. Dorcas Yole is an Associate Research Scientist at the Institute of Primate Research. Before joining TU-K, she was a Senior Research Scientist at Institute of Primate Research (IPR), a biomedical research centre, where she served as the Chair of Parasitology Department and also Chair of the Institutional Scientific and Ethical Review Committee

She has been a reviewer for National Commission of Science, Technology and Innovation; and she is a reviewer for the National Research Fund. She is a Trainer of Trainers for World Health Organization (WHO) Good Laboratory Practice, and also a Trainer of Trainers for WHO Effective Project Planning and Evaluation for Biomedical Research. Her major areas of research are: Vaccine development, Drug and Molluscicide development for Schistosomiasis intervention. Prof Dorcas Yole is well published and has contributed to 8 World Health Organization Manuals/Handbooks.

Dr. Bobadoye Ayodotun is the Chief Operating Officer (COO) of the Global Emerging Pathogens Treatment Consortium (GET). He has a B.Sc. Animal Science (University of Ibadan, Nigeria), M. Tech, Animal Production and Health (Federal U niversity of Technology, Akure, Nigeria), Executive Masters Project Management (Project Management College London) and PhD Climate Change and Adaptation (Institute for Climate Change and Adaptation, University of Nairobi, Kenya). He has over 15 years research and teaching experience with African Technology and Policy Studies Network, Nairobi, Kenya (ATPS) and He is a scholar of the Woodrow Wilsonnternational Center for Scholars, Washington, DC; and also, a

Scholar of Africa Science Service Center on Climate Change and Adapted Land Use (WASCAL). Dr. Bobadoye has led many internationally funded research projects bordering on climate change, natural resource management, science, technology and innovation (STI); innovation systems; development issues; policy development, analysis and advocacy; epidemiology; biosecurity and private sector engagements. He is a member of many professional organizations and has published over 50 journal articles in reputable journals.

Dr. Afolabi Muhammed is a Global Health Scientist and UKRI Fellow at the London School of Hygiene & Tropical Medicine, UK. He obtained a medical degree from the University of Ibadan; a master's degree in Public Health from Obafemi Awolowo University, both in Nigeria and a PhD in Clinical Research from the London School of Hygiene & Tropical Medicine, UK. He is also a Fellow of West African College of Physicians and National Postgraduate College of Nigeria in Family Medicine, as well as the UK Higher Education Academy. Dr Afolabi has worked extensively on the clinical vaccine trials related to the control and prevention of Ebola, HIV and malaria across several African countries. He led the Ebola paediatric vaccine trials in Sierra Leone, findings of which contributed to the approval of the novel two-dose Ebola vaccine regimen (ZABDENO/MVABEA) by the European Medicines Agency. His other research interests include bioethics issues shaping the conduct of clinical trials in vulnerable populations. Dr Afolabi currently serves on several vaccines and immunisations committees including WHO Strategic Advisory Group of Experts (SAGE) Working Group on COVID-19 vaccines.

Dr. Babatunde A. Saka is a public health specialist with special attention on molecular epidemiology and prediction. Dr Saka graduated from the University of Ibadan with a Doctor of Veterinary Medicine degree where he also completed his Master of Science and PhD in Preventive Veterinary Medicine. He worked in the private sector until 2011 when he was appointed as a Research and Teaching Assistant for the Department of Veterinary Public Health and Preventive Medicine in the University of Ibadan. He served in this capacity as a clinical instructor, project design and monitoring as well as research assistant to the leading aquatic epidemiologist and toxicologist in the university for five years. Dr Saka presently works with the GET Consortium as the Project, and he currently serves as a technical consultant on Biosecurity and One Health as well as the Secretary of the Data Safety and Monitoring Board to Lagos State Ministry of Health. He is a member of the Lagos State Biosecurity and Biobanking Governing Council, Nigerian Biological Safety Association, Genetic Toxicologist Association of Nigeria, Nigerian Veterinary Medical Association and International Federation of Biosafety Associations. He is serving as the laboratories coordinator as well as the Deputy Incident Manager for Lagos State Covid Response. His hobbies include reading and watching movies especially epics.

Prof. Olanike Kudirat Adeyemo is a Nigerian professor of Veterinary Public Health and Preventive Medicine at University of Ibadan. She is the current Deputy Vice Chancellor (research, innovation and strategic partnership), the first person to attain the role at the University. Her research areas are on Aquatic toxicology, Aquatic veterinary medicine and fish food safety. She is the first female veterinarian to be inducted into the African Academy of Sciences and the Nigerian Academy of Science. Prof. Olanike's research is focused on Aquatic and Wildlife Epidemiology and Toxicology, Food Safety and Global Public Health. In 2011, Adeyemo was appointed an epidemiological and toxicological expert on the Joint FAO/WHO Expert Committee (JECFA).

In 2019, she was named a Fellow of The World Academy of Sciences for the advancement of science in developing countries, a Fellow at the Society for Environmental Toxicology and Pollution Mitigation. In 2016, she was named a Fellow of the Nigerian Academy of Science. In 2012, she was named a Fellow of the African Academy of Sciences. In 2010, she was named a Fellow of the African Scientific Institute (California, USA) and listed in ASI's 2011 edition of "Black Achievers in Science and Technology. In 2007 she was named a Fellow of the Eisenhower Fellowship Program and in 2002 she was named a Fellow of the Leadership for Environment and Development program in the UK.

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Spatial Monthly Distribution of Malaria Parasite and Anopheles Mosquitoes in Minna, Niger State, Nigeria

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ABSTRACT

Malaria is a life-threatening mosquito-borne tropical disease that continues to pose public health challenges in Nigeria. This study was conducted to evaluate the spatial monthly and seasonal distribution of *Anopheles* mosquito in Minna, Niger State, Nigeria. Mosquitoes were sampled using the Pyrethroid Spray Catch (PSC) technique and were identified morphologically using keys. A total of 3303 (100%) mosquito vectors were captured between June 2016 and May 2017. The total number of mosquitoes captured comprised, 791(23.95%) Anopheline and 2512(76.05%) Culicine. The highest number of mosquito vectors were captured in May 2017 with 528(15.99%) followed by 453(13.71%) in August 2016, while the least of 78(2.36%) were captured in November 2016 ($P<0.05$). Out of the 791(23.95%) *Anopheles* mosquitoes captured, the highest being 233 (44.13%) was recorded in May 2017. Seven (7) species of female *Anopheles* mosquitoes were encountered during the study period, namely: *Anopheles gambiae* (53.22%), *Anopheles funestus* (18.46%), *Anopheles squamosus* (4.55%), *Anopheles moucheti* (5.07%), *Anopheles coustani* (9.23%), *Anopheles nili* (7.21%) and *Anopheles pharoensis* (2.28%). This study revealed a high distribution of *Anopheles* mosquitoes in the study areas. The results of this study would be useful in planning an effective site-specific malaria vector control, as it highlights the need to give special consideration to the predominance of a single malaria vector, *Anopheles gambiae* in Minna, Niger State.

Keywords: Pyrethroid; *Anopheles*; Predominance; Minna; Niger State.

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INTRODUCTION

Malaria is a mosquito-borne disease in humans and animals [1, 2]. It is caused by parasitic protozoans of the genus *Plasmodium* with species P. *falciparum,* P.*vivax,* P*. malariae,* P*. knowlesi,* and P. *ovale.* The mosquitoes which act as vectors of this disease are female *Anopheles funestus, Anopheles moucheti, Anopheles gambiae, Anopheles arabiensis,* and other *Anopheles* species [3, 4].

Nigeria suffers the world's greatest malaria burden, with approximately 51 million cases and 207,000 deaths reported annually (approximately 30 % of the total malaria burden in Africa), while 97 % of the total population (approximately 173 million) is at risk of infection according to World Health Organization [5, 6, 7]. Moreover, malaria accounts for 60 % of outpatient visits to hospitals and leads to approximately 11 % maternal mortality and 30 % child mortality, especially among children less than 5 years old [5, 8]. Among the malaria parasites, *P. falciparum* is the most fatal species and exhibits complex genetic polymorphism which may explain its ability to develop multiple drug resistance and circumvent vaccines [9, 10, 11].

In Nigeria, malaria in pregnant women is a major public health concern because it is the major cause of maternal mortality. The major complications in pregnant women resulting from malaria are low birth weight in newborn babies, high placental plasmodia burden, foetal complications, and sometimes newborn death [12, 13].

Malaria is a fatal insect-borne tropical disease that continues to pose public health challenges with about 3.3 billion people at risk of being infected by the disease globally in 2023 [5]. This, however, was reduced to 3.2 billion people in 2015 probably as a direct result of the several global control efforts that eliminated malaria in some countries [6]. Africa carries the greatest burden of disease and it is one of the major causes of morbidity and mortality [14]. Globally, approximately 214 million cases of malaria occur annually [6] and 438,000 deaths were attributed to malaria in 2023, particularly in sub-Saharan Africa, where an estimated 90 % of all malaria deaths occur [6].

Malaria has been around for thousands of years and is still a major problem today. Despite efforts to eradicate malaria over the past 100 years, 149–274 million cases and 537,000–907,000 deaths from malaria occur in sub-Saharan Africa each year [15, 16]. Species of *Plasmodium* can infect reptiles, birds, and mammals. Of the more than 100 *Plasmodium* species, there are five (5) that infect humans. The transmission of the disease from one human to another involves mosquitoes of the genus *Anopheles* [17]. *Anopheles gambiae* is the principal vector of malaria in sub-Saharan Africa [18, 19] where more than 90% of the World's clinical cases are recorded [20, 21, 22]. According to recent World Health Organization's reports and statistics, malaria threatens the life and health status of about two-thirds of the world's human population, resulting in as much as 600 million clinical attacks and an estimated one million deaths annually [6]. The disproportionately high intensity of malaria transmission in sub-Saharan Africa is due to the widespread distribution and high vectorial capacity of the primary vector, i.e., *An. gambiae* in the region [23, 24]. Studies have established this anopheline species as one of the most efficient transmitters of *Plasmodium* parasites in the world. The epidemiological success of *An. gambiae* is largely dependent on its highly dynamic ecological behaviour [25] that has evolved over a long time due to certain tropical weather conditions that promote mosquito proliferation and human/vector contact.

Although, *An. gambiae* is widely distributed in sub-Saharan Africa, its behaviour and ecological adaptability vary considerably from one locality to another, partly dictated by spatio-temporal differences in seasonal weather conditions [26, 27]. Such temporal variations in anopheline vector behaviour, in response to seasonal changes in weather conditions in an area, are responsible for enormous heterogeneity in the intensity of malaria transmission and the efficacy of control measures [28]. Studies have shown that in the rainy season, anopheline mosquitoes tend to be more endophagic, endophilic and anthropophagic, to avoid the harsh ecological conditions outdoors [29, 30, 31]. Also, these mosquitoes breed more in natural larval habitats, such as temporary sunlit ground pools in the rainy season due to the high proliferation of such sites during the period which guarantee faster developmental and higher survival rates [32]. The local interactions of combinations of these important entomological drivers of malaria transmission, occasioned by behavioural responses of anopheline mosquitoes to prevailing weather conditions, will go a long way in determining vectorial efficiency and hence, the patterns of malaria transmission, as well as the efficacy of implemented vector-control measures. Also, residual indoor spraying with insecticides such as Pyrethroids and the use of insecticide-treated bed nets are more effective at controlling malaria vectors when vectors prefer to feed and rest indoors [33].

METHODOLOGY

Study Area

The study was conducted in Minna, Niger State, Nigeria. Minna is bounded to the north by Kebbi, Sokoto, Kaduna, and Federal Capital Territory (FCT) to the east, Benin Republic to the west, and Kwara and Kogi to the south.

The metropolis spreads across two Local Government Areas (LGAs) namely: Bosso and Chanchaga. The mean annual rainfall of Minna is 1334 mm with August and September recording the highest monthly rainfall of about 300 mm. The highest

monthly temperature is recorded in March with an average daily temperature of 30°C and the lowest in August at about 22°C. Minna has a tropical wet and dry climate with a pronounced dry season. Ten (10) different locations were randomly selected, five (5) each from Bosso and Chanchaga LGAs. These study locations include Bosso Estate, Tudun Fulani, Rafin Yashi, Dutsen Kura, Shanu Village, Maikunkele, Kpakungu, Tunga, Maitumbi and Chanchaga (Table 1).

Table 1: Global Positioning System (GPS) coordinates of the study sites

S/No	Study Sites	Latitude	Longitude
1	Bosso Estate	9°38'52"N	6°32'35"E
2	Tudun Fulani	9°38'41"N	6°32'31"E
3	Rafin Yashi	9°38'56"N	6°32'50"E
4	Shanu Village	9°36'50"N	6°32'01"E
5	Maikunkele	9°36'48"N	6°32'49"E
6	Tunga	9°38'24"N	6°32'29"E
7	Dusten Kura	9°35'32"N	6°31'48"E
8	Kpakungu	9°38'36"N	6°32'24"E
9	Maitumbi	9°37'44"N	6°34'40"E
10	Chanchaga	9°39'13"N	6°32'20"E

Each of these houses was chosen based on the condition of windows, doors, and walls before they were examined, and whether the houses had closed or open eaves. The surroundings of the households were examined and any nearby water bodies, cattle sheds, or other animal sheds noted. Other environmental variables recorded included whether the houses were located at the edge or in the middle of the villages and whether people kept animals, such as cattle, pigs or chickens.

Pyrethrum Spray Collection (PSC)

A total of 10 houses per LGA per month were sampled using the Pyrethrum Spray Collection (PSC) method as described by WHO (2005, 2008) to sample indoor-resting mosquitoes. The houses were sampled by two persons, using an aerosol insecticide (Baygon) containing the active ingredients of 0.05 percent Imiprothrin, 0.05 percent Prallethrin, and 0.015 percent Cyfluthrin.

Before spraying, the floors were covered with clean white bed sheets, outlets were closed, and the two sprayers began spraying as they moved in opposite direction, spraying inside the room after which the door was closed for 15 minutes and then opened so the sprayers would enter and collect mosquitoes. Mosquitoes that were knocked down were collected from the white cloth that was laid down before spraying. The spraying was done between 6:00 am and 9:00 am.

The mosquitoes were collected using feather-weight forceps and then placed in Petri dishes or paper cups containing a damp filter paper. Anopheline mosquitoes were preserved on damp absorbent paper in a cool box, transported to the Department of Animal Biology Laboratory, Federal University of Technology, Minna, Niger State and later identified to the species level by morphological criteria [18, 34, 35, 36].

Morphological Identification of Mosquito Samples

All mosquitoes collected were identified and sorted out under a stereomicroscope (Leica model NSW series IMNS 210) and Olympus Tokyo VT-II 225329 Entomological microscope. All mosquitoes were identified using the morphological keys of Gillies and De Meillion [34], and Gillies and Coetzee [18] by sex and whether they were anophelines or culicines.

Data Analysis

Data generated were analyzed using the SPSS software version 20.0 and Excel package. The monthly spatial distribution patterns of the malaria vector species and the Culicine genera, i.e. *Culex* spp. mosquitoes were presented graphically. The graph was also used to represent the environmental data obtained from the study areas. The relationship between the seasonal relative abundance of mosquito vector individuals in the study was evaluated using Chi-Square analysis. The relationship between *Anopheles* species and months/season was analyzed using one-way ANOVA. Statistical analyses on abundance data of predominant species and environmental variables to species distributions were analyzed. Pearson correlation coefficients were computed for the dominant species using SPSS 20.0 to assess the correlations between mosquito abundance and environmental variables.

RESULTS

This study established that seven (7) species of *Anopheles* mosquitoes were encountered during the study period; *Anopheles gambiae, An. funestus, An. squamosus, An. moucheti, An. coustani, An. nili* and *An. pharoensis*. The highest *Anopheles* mosquito species population was recorded in May. The distribution of *Anopheles* mosquito species varies significantly (P<0.05) monthly. *Anopheles gambiae* was the dominant species throughout the study period while the least was *An. pharoensis*. The highest number of *An. gambiae, An. funestus, An. coustani*, and *An. nili*, were recorded in May, while *An. squamosus* was highest in August, *An. moucheti* and *An. Pharoensis* was highest in April.

Monthly relative abundance of mosquito vector individuals in Minna

A total of three thousand, three hundred and three (3303) mosquito vectors were sampled between the period of June 2016 and May 2017. Of the 3303 mosquitoes sampled, 791 (23.95%) were Anopheline while 2512 (76.05%) were Culicine. The highest number of mosquitoes were sampled in May, 2017 528 (15.99%), followed by 453 (13.71%) in August 2016 while the least number of 78 (2.36%) mosquitoes were sampled in November 2016. Out of the 791 (23.95%) Anopheline mosquitoes sampled, the highest number of 233 (44.13%) was recorded in May 2017, followed by August 2016 with 111 (24.50%) mosquitoes while the least was recorded in November 2016 with 8 (10.26%) mosquitoes. Conversely, of the 2512 (76.05%) Culicine mosquitoes sampled, the highest number of 350 (80.46%) mosquitoes were sampled in June 2016, followed by August 2016 with 342 (75.50%) mosquivtoes while the least numbers of Culicine mosquitoes 70 (89.74%) were sampled in November 2016. There was a statistically significant (p<0.05) difference in the monthly relative abundance of mosquito vectors in Bosso and Chanchaga Local Government Areas (Figure 2).

Seasonal relative abundance of mosquito vector individuals in Minna

On seasonal basis, a total of 1209 (36.60%) mosquito vectors were sampled in the dry season period while 2094 (63.40%) mosquitoes were sampled during the rainy season period. Of the 1209 (36.60%) mosquitoes collected during the dry season period, 257 (21.26%) were Anopheline while 952 (78.74%) were Culicine. In the same vein, a total of 534 (25.50%) Anopheline and 1560 (74.50%) Culicine mosquitoes were collected during the rainy season period. The relative abundance of mosquito vector genera varies significantly (P<0.05) based on the two seasons in Bosso and Chanchaga Local Government Areas as shown in Table 2. The environmental data collected and populations of Anopheles mosquitoes collected are shown in Table 3. Correlation analysis also determined the relationship between *Anopheles* mosquito species and the environmental data (Supplementary Table 1). The record showed that the abundance of *Anopheles* mosquito species in the study area is dependent on rainfall and temperature. Certainly, rainfall and temperature directly affect mosquito breeding, survival, behavior and malaria transmission. More so, the development and survival rates of both the *Anopheles* mosquitoes and the *Plasmodium* parasites that cause malaria depend on temperature, making this a potential driver of mosquito population dynamics and malaria transmission. There was a significant correlation between *Anopheles* mosquito species and the environmental data collected.

Table 2: Seasonal Relative Abundance of Mosquito Vector Genera in Minna

Seasons	Anopheline (%)	Culicine (%)	Total (%)
Dry season	257 (21.26)	952 (78.74)	1209 (36.60)
Rainy season	534 (25.50)	1560 (74.50)	2094 (63.40)
Total	**791 (23.95)**	**2512 (76.05)**	**3303 (100)**

χ^2 Cal = 7.58; χ^2 tab = 3.84; df = 1

Table 3: Environmental Data collected and *Anopheles* mosquitoes Population.

		Environmental Data		
Months	***Anopheles* species**	**Relative Humidity (%)**	**Temperature (°C)**	**Rainfall (mm)**
January	70	81.2	27.8	1.4
February	12	81.8	29.8	5.4
March	35	80.6	27.4	11.9
April	102	83.0	27.3	60.9
May	233	84.0	25.4	153.9
June	85	86.3	25.2	174.5
July	48	88.2	24.3	206.6
August	111	85.0	25.4	261.7
September	21	78.1	27.2	234.9
October	36	73.2	28.5	96.4
November	8	70.3	28.0	1.1
December	30	64.5	29.2	0.3
Total	**791**	**956.3207**	**325.4908**	**1209**
Average		79.7	27.12	100.75

Monthly distribution of *Anopheles* mosquito species in Minna

The monthly distribution of *Anopheles* mosquito species in Bosso and Chanchaga Local Government Areas is presented in Table 4. Seven (7) species of *Anopheles* mosquito were encountered during the study period; *Anopheles gambiae complex, An. funestus, An. squamosus, An. moucheti, An. coustani, An. nili* and *An. pharoensis*. The highest *Anopheles* mosquito species population recorded in May was 116.50±24.75 (29.46%). The distribution of *Anopheles* mosquito species varies significantly (P<0.05) monthly. *Anopheles gambiae* complex was the dominant 210.50±47.35 (53.22%) species throughout the study period while the least was *An. pharoensis* with 9.00±5.66 (2.28%). The highest number of *An. gambiae* complex*, An. funestus, An. coustani*, and *An. nili* (74.00±15.56, 17.50±2.12, 10.50±2.12 and 6.50±2.12) respectively, were recorded in May, while *An. squamosus* was highest (7.00±1.41) in August, *An. moucheti* and *An. pharoensis* (5.50±2.12, 2.00±1.41) respectively were highest in April.

ANOVA analysis showed that there was no statistically significant difference (p>0.05) between and within the *Anopheles* species encountered during the study period (Supplementary Table 2).

Spatial monthly and seasonal distribution of *Anopheles* mosquitoes in Minna

The spatial monthly distribution of *Anopheles* mosquitoes in Bosso and Chanchaga Local Government Areas is presented in Table 5. The *Anopheles* mosquito population encountered in the study areas varied significantly (P<0.05) among the months of collection and between the seasons. Throughout the collection period and in the sampling locations, the highest *Anopheles* mosquito population was recorded in the month of May except for Maikunkele and Tunga where the highest *Anopheles* mosquito population was recorded in the month of August and April, respectively. As the collection moved down from January to February, the *Anopheles* mosquito population collected fell significantly (P<0.05), thereafter the *Anopheles* mosquito population sampled increased from March to May, where the highest *Anopheles* mosquito population was observed. However, in most of the sampling areas, after the month of May, the *Anopheles* mosquito population encountered fell drastically/significantly (P<0.05). Additionally, the highest percentage distribution was recorded in the month of May (29.46%) while the least was recorded in the month of November (0.88%).

Seasonally, *Anopheles* mosquito distribution varied likewise significantly, and the *Anopheles* mosquito population was higher in the rainy season 267.00±96.15 (67.51%) than in the dry season 128.00±54.45 (32.36). This is not the same for *Anopheles* mosquitoes sampled in Tunga and Kpakungu where no significant difference (P>0.05) was observed in the mosquito distribution for the two seasons

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Table 2: Monthly Distribution of *Anopheles* Mosquito Species

Months/An. species	*An. gambiae* complex	*An. funestus*	*An. Squamosus*	*An. moucheti*	*An. coustani*	*An. Nilli*	*An. pharoensis*	Total (%)
June	$24.50\pm2.12_{d}^{d}$	$6.50\pm2.12_{c}^{b}$	$3.00\pm1.41_{b}^{b}$	$2.50\pm0.71_{ab}^{ab}$	$3.50\pm2.12_{b}^{bc}$	$1.50\pm0.71_{a}^{abc}$	$1.00\pm1.41_{a}^{ab}$	42.50±10.6 (10.75)
July	$10.50\pm3.53_{c}^{abc}$	$4.50\pm2.12_{b}^{ab}$	$2.50\pm0.71_{b}^{b}$	$1.00\pm0.00_{b}^{a}$	$2.00\pm1.41_{b}^{abc}$	$3.50\pm2.12_{b}^{bcd}$	$0.00\pm0.00_{a}^{a}$	24.00±9.89 (6.07)
August	$20.50\pm2.12_{e}^{bcd}$	$12.50\pm1.12_{d}^{c}$	$7.00\pm1.41_{c}^{c}$	$1.00\pm1.41_{a}^{a}$	$9.00\pm1.41_{c}^{d}$	$4.50\pm0.71_{b}^{de}$	$1.00\pm0.00_{a}^{ab}$	55.50±8.18 (14.03)
September	$5.50\pm2.12_{c}^{a}$	$2.50\pm2.12_{b}^{ab}$	$0.00\pm0.00_{a}^{a}$	$0.50\pm0.71_{a}^{a}$	$1.00\pm1.41_{ab}^{ab}$	$0.50\pm0.71_{a}^{a}$	$0.50\pm0.71_{a}^{ab}$	10.50±7.78 (2.65)
October	$8.00\pm1.41_{d}^{ab}$	$5.50\pm2.12_{c}^{b}$	$0.00\pm0.00_{a}^{a}$	$0.50\pm0.71_{a}^{a}$	$3.00\pm1.41_{b}^{abc}$	$1.00\pm0.00_{ab}^{ab}$	$0.00\pm0.00_{a}^{a}$	18.00±5.56 (4.55)
November	$2.00\pm1.41_{b}^{a}$	$1.00\pm1.41_{ab}^{a}$	$0.00\pm0.00_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	$0.50\pm0.71_{a}^{ab}$	$0.50\pm0.71_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	4.00±4.24 (1.01)
December	$9.50\pm3.53_{c}^{abc}$	$2.50\pm0.71_{b}^{ab}$	$0.50\pm0.00_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	$2.00\pm1.41_{b}^{abcd}$	$0.50\pm0.71_{a}^{ab}$	15.00±6.36 (3.79)
January	$19.50\pm4.95_{d}^{bcd}$	$6.00\pm1.41_{c}^{b}$	$0.00\pm0.00_{a}^{a}$	$4.50\pm2.12_{c}^{bc}$	$1.50\pm0.71_{b}^{ab}$	$2.00\pm0.00_{b}^{abcd}$	$1.50\pm0.71_{b}^{ab}$	35.00±9.90 (8.85)
February	$3.00\pm1.41_{b}^{a}$	$1.00\pm0.00_{a}^{a}$	$0.50\pm0.71_{a}^{a}$	$0.50\pm0.71_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	$1.00\pm1.41_{a}^{ab}$	$0.00\pm0.00_{a}^{a}$	6.00±4.24 (1.52)
March	$11.00\pm2.83_{c}^{abc}$	$3.00\pm1.41_{b}^{ab}$	$0.50\pm0.71_{a}^{a}$	$0.00\pm0.00_{a}^{a}$	$0.50\pm0.71_{a}^{ab}$	$1.50\pm0.71_{ab}^{abc}$	$1.00\pm0.00_{ab}^{ab}$	17.5±6.37 (4.42)
April	$22.50\pm6.36_{d}^{cd}$	$10.50\pm2.12_{c}^{c}$	$1.50\pm0.71_{b}^{ab}$	$5.50\pm2.12_{b}^{c}$	$5.00\pm1.41_{b}^{c}$	$4.00\pm1.41_{b}^{cde}$	$2.00\pm1.41_{a}^{b}$	51.00±15.54 (12.90)
May	$74.00\pm15.56_{e}^{e}$	$17.50\pm2.12_{d}^{d}$	$2.50\pm0.71_{a}^{b}$	$4.00\pm1.41_{b}^{bc}$	$10.50\pm2.12_{c}^{d}$	$6.50\pm2.12_{b}^{e}$	$1.50\pm0.71_{a}^{ab}$	116.50±24.75 (29.46)
Annual	**210.50±47.35 (53.22)**	**73.00±18.78 (18.46)**	**18.00±6.37 (4.55)**	**20.00±9.90 (5.07)**	**36.50±13.42 (9.23)**	**28.50±12.02 (7.21)**	**9.00±5.66 (2.28)**	**395.50±113.5 (100)**

Values with the same superscript within a row are not significantly different at P>0.05
Values with the same subscript within a column are not significantly different at P>0.05

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Table 3: Spatial Monthly Distribution of *Anopheles* Mosquitoes in Minna

	BES	TDF	RFY	SHAV	MAIK	TUN	DUT	KPA	MAIT	CHA	**Aggregate**
Jun	4.50±2.12$_{c}^{a}$	7.50±2.12$_{cd}^{d}$	3.00±1.41$_{bc}^{ab}$	8.50±2.12$_{c}^{b}$	2.50±0.71$_{b}^{ab}$	1.50±0.71$_{a}^{ab}$	3.00±1.41$_{bc}^{a}$	1.00±1.41$_{a}^{a}$	3.50±0.71$_{bc}^{b}$	7.50±2.12$_{b}^{d}$	42.50±14.84
Jul	2.50±0.71$_{b}^{b}$	0.50±0.71$_{a}^{a}$	2.00±0.00$_{b}^{abc}$	2.00±1.41$_{b}^{a}$	2.50±0.71$_{b}^{ab}$	1.00±1.41$_{a}^{ab}$	5.50±2.12$_{c}^{de}$	4.50±2.12$_{bc}^{a}$	2.50±0.71$_{b}^{ab}$	1.00±1.41$_{a}^{a}$	24.00±11.31
Aug	5.00±1.41$_{c}^{c}$	5.50±2.12$_{bc}^{c}$	3.00±1.41$_{b}^{abc}$	7.50±2.12$_{d}^{b}$	10.00±1.41$_{e}^{c}$	3.00±1.41$_{b}^{bc}$	2.00±1.41$_{a}^{ab}$	1.50±0.71$_{a}^{a}$	11.50±2.12$_{e}^{c}$	6.50±2.12$_{b}^{d}$	55.50±16.24
Sept	5.00±1.41$_{c}^{c}$	0.50±0.71$_{a}^{b}$	0.50±0.71$_{b}^{a}$	0.00±0.00$_{a}^{a}$	1.00±0.00$_{b}^{a}$	1.00±1.41$_{b}^{ab}$	0.50±0.71$_{b}^{ab}$	0.50±0.71$_{b}^{a}$	1.00±0.00$_{b}^{ab}$	0.50±0.71$_{b}^{a}$	10.50±6.37
Oct	1.50±0.71$_{b}^{a}$	5.00±2.83$_{bc}^{d}$	0.50±0.71$_{ab}^{a}$	1.50±0.71$_{b}^{a}$	0.00±0.00$_{a}^{a}$	1.50±0.71$_{b}^{ab}$	2.50±0.71$_{ab}^{a}$	1.00±0.00$_{ab}^{a}$	1.50±0.71$_{b}^{ab}$	3.00±1.41$_{b}^{a}$	18.00±8.50
Nov	0.50±0.71$_{b}^{a}$	0.00±0.00$_{a}^{a}$	0.50±0.71$_{b}^{a}$	0.00±0.00$_{a}^{a}$	0.00±0.00$_{a}^{a}$	1.00±0.00$_{b}^{ab}$	0.00±0.00$_{a}^{a}$	0.50±0.71$_{b}^{a}$	0.50±0.71$_{b}^{a}$	0.50±0.71$_{b}^{a}$	3.50±3.55
Dec	2.50±0.71$_{b}^{b}$	0.50±0.71$_{a}^{a}$	0.50±0.71$_{b}^{a}$	1.50±0.71$_{a}^{a}$	2.00±0.00$_{ab}^{a}$	1.00±1.41$_{a}^{ab}$	0.50±0.71$_{a}^{ab}$	2.00±0.00$_{ab}^{a}$	2.00±0.00$_{ab}^{ab}$	3.00±0.71$_{b}^{a}$	15.50±5.67
Jan	4.50±2.12$_{c}^{c}$	2.00±1.41$_{ab}^{a}$	4.00±2.83$_{c}^{bc}$	1.50±0.71$_{a}^{a}$	3.00±0.00$_{b}^{ab}$	4.00±1.41$_{c}^{c}$	4.00±1.41$_{c}^{e}$	6.50±0.71$_{d}^{d}$	3.50±2.12$_{bc}^{b}$	1.50±2.12$_{a}^{a}$	34.50±14.84
Feb	0.50±0.71$_{ab}^{a}$	1.00±0.00$_{b}^{a}$	1.00±1.41$_{b}^{ab}$	1.00±0.00$_{b}^{a}$	0.00±0.00$_{a}^{a}$	0.00±0.00$_{a}^{a}$	1.50±0.71$_{c}^{b}$	0.50±0.71$_{ab}^{a}$	0.00±0.00$_{a}^{a}$	0.50±0.71$_{ab}^{a}$	6.00±4.25
Mar	1.00±0.00$_{a}^{a}$	2.00±1.41$_{ab}^{b}$	2.50±0.71$_{b}^{b}$	1.00±1.41$_{a}^{a}$	1.50±0.71$_{a}^{a}$	2.00±0.00$_{b}^{ab}$	1.00±1.41$_{a}^{a}$	3.50±2.12$_{c}^{ab}$	2.00±141$_{b}^{ab}$	1.00±0.00$_{a}^{a}$	17.50±9.18
Apr	4.00±1.41$_{d}^{b}$	6.50±2.12$_{c}^{c}$	4.50±2.12$_{d}^{bc}$	8.50±2.12$_{e}^{b}$	5.00±1.41$_{d}^{b}$	11.50±2.12$_{f}^{e}$	3.50±2.12$_{c}^{b}$	2.50±2.12$_{b}^{ab}$	3.50±0.71$_{ab}^{b}$	1.50±0.71$_{a}^{a}$	51.00±16.96
May	16.0±11.31$_{bc}^{b}$	10.50±2.12$_{b}^{d}$	13.00±1.411$_{d}^{b}$	8.50±2.12$_{ab}^{b}$	8.50±3.54$_{a}^{c}$	8.50±0.71$_{a}^{d}$	6.50±0.71$_{a}^{e}$	8.00±4.24$_{a}^{c}$	15.50±2.12$_{bc}^{d}$	21.50±10.61$_{d}^{c}$	116.50±38.89
Annual	**47.50±23.33**	**41.50±16.26**	**35.00±14.14**	**41.50±13.43**	**36.00±8.49**	**36.00±11.30**	**30.50±13.43**	**32.00±15.56**	**47.00±11.32**	**48.00±23.34**	**395.50±150.6**

Values with the same superscript within a row are not significantly different at P>0.05
Values with the same subscript within a column are not significantly different at P>0.05
Keys: BES = Bosso Estate, TDF = Tudun Fulani, RFY = Rafin Yashi, SHAV = Shanu Village, MAIK = Maikunkele, TUN = Tunga
DUT = Dutsen Kura, KPA = Kpakungu, MAIT = Maitumbi and CHA = Chanchaga

DISCUSSION

In this study, two mosquito vector genera were encountered. A total of 3303 (100%) mosquitoes consisting of Anopheline 791 (23.95%) and Culicine 2512 (76.05%). However, the results of this study are higher than that reported by Olayemi *et al*. [47]. Seven (7) species of *Anopheles* mosquito were encountered during the study period; *Anopheles gambiae complex, An. funestus, An. squamosus, An. moucheti, An. coustani, An. nili* and *An. pharoensis*.

Based on monthly collection, mosquito vectors were most abundant in the month of May for Anopheline species (44.13%) and June for Culicine (80.46%). There was no positive relation between the abundance of mosquito vectors with rainfall and the majority of the anopheline species. We did not find any significant correlation between rainfall, and the known malaria vector; *An. gambiae complex*. It may be because of the association with rice fields and irrigated cropland, where the females deposit their eggs on moist soil [37]. Also, Rahman *et al*. [38] did not find a correlation between mosquito vector abundance and rainfall in Malaysia. The observed diversity of species in this study may be due to favourable breeding sites in the study area. Ye' *et al.* [39] and Thomson *et al.* [40] reported that soil moisture is a major factor affecting the abundance of some species. However, measurements of soil moisture were not included in this study.

We did not find any significant association with temperature, mosquito density and malaria incidence in our study. Certainly, the temperature directly affects mosquito breeding, survival, and behavior and malaria transmission as earlier reported [41]. We were unable to detect a significant relationship with this factor because the temperature ranges in this region are always suitable for mosquito breeding and development. Moreover, statistical significance alone does not always explain the complex biological dynamics of mosquitoes and temperature.

The result of this study is similar to the report of Atting and Akpan [42] in Uyo, Nigeria, where *Anopheles* mosquitoes were most abundant between the months of May to October. More so, mosquito vectors were more abundant in the rainy season (63.40%) than in the dry season (36.40%). This seasonal variation in the mosquito vector genera abundance was also documented by Mgbemena et al. [43].

The highest *Anopheles* mosquito population was recorded in the month of May except in Maikunkele and Tunga where the highest *Anopheles* mosquito population was recorded in the month of August and April, respectively. Environmental and climatic factors could play a role but most vital is the availability of host settlement. The reason why a higher mosquito population was recorded in the months of August and April in Tunga and Maikunkele could be due to the onset of rains that the mosquito eggs hatch immediately due to the aestivation period, while in August could be due to the availability of suitable breeding sites created by the rains. In the results section, it was mentioned that in Tunga and Kpakungu no significant difference (P>0.05) was observed in the mosquito distribution for the two seasons. This could be due to the settlement within the locations and the availability of breeding sites in those areas.

Anopheles mosquito relative abundance was moderately high (23.95%) in the study areas, although, the use of some insecticides and the usage of Long Lasting Insecticidal Treated Nets can reduce vector infectivity as well as vector survival rate and the length of the sporogonic cycle [44]. This result revealed that *Anopheles* mosquito abundance was high in Bosso Estate (47.50±23.33), Maitumbi (47.00±11.32) and Chanchaga (48.00±23.34). This could be due to the availability of temporary breeding sites and favourable environmental factors such as rainfall and temperature as earlier reported by Anumudu *et al.* [45] and Paaijmans *et al.* [46]. Some of the environmental practices within the study areas include disposing of containers, receptacles, water storage jars, unused tyres, abandoned cans etc. also play a major role in the distribution of *Anopheles* mosquitoes in the study areas [27, 30, 47]. Also, the *Anopheles* mosquito encountered in this study area was reported by Coluzzi *et al.* [48] as the most important vector of the malaria parasites in the sub – Saharan Africa, in connection with certain climatic factors most especially the annual precipitation that appears to influence the range and the relative abundance of *Anopheles* mosquitoes.

Interestingly, a similar result was earlier reported by Omalu *et al.* [49] who recorded a high relative abundance of *Anopheles* mosquitoes in the Gidan Kwano and Maikunkele areas of Minna, Niger state, Nigeria. However, a low relative abundance of *Anopheles* mosquitoes was recorded in Rafin Yashi (35.00±14.14), Maikunkele (36.00±8.49), Tunga (36.00±11.30), Dutsen Kura (30.30±13.43) and Kpakungu (32.00±15.56). This could be because of over-flooding of the temporary breeding sites during the peak of the rainy season when most of the mosquito larvae are washed away [42, 50].

CONCLUSION

This study demonstrates that the spatial distribution of *Anopheles* mosquitoes all year-round in Minna was due to the favourable environmental conditions. Therefore, an integrated vector management system covering year-round should be adopted to reduce malaria morbidity and mortality in Minna.

We recommend that vector ecologists cautiously consider the complex nature of the relationship between malaria vectors and climate variables. Detailed studies

of vector bionomics, continuous monitoring, and malaria transmission dynamics are essential for predicting outbreaks of disease and, if necessary, control of the vector mosquitoes in Minna, Niger State, Nigeria.

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance
CDC: Centre for Disease Control and Prevention
DNA: Deoxyribonucleic Acid
FCT: Federal Capital Territory
FMOH: Federal Ministry of Health
PCR: Polymerase Chain Reaction
PSC: Pyrethrum Spray Collections
WHO: World Health Organization
LGAs: Local Government Areas

ETHICAL APPROVAL

The study protocol was approved by the Niger State Ministry of Health (Niger State Hospital Management Board) (HMB/GHM/STA/136/VOL.III/440). Individual informed consent was also obtained from all the participants after the aim and objectives of the study were fully explained to them. Verbal consent was also taken from Fall the household heads for the study because the majority of them were illiterate.

CONFLICT OF INTEREST

The authors declared that they have no competing interests.

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A Cross-Sectional Study of Antimicrobial Resistance Pattern Across Two States in Nigeria

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ABSTRACT

Antimicrobial resistance has been declared by the World Health Organization as a threat to countries and across various sectors. This study aimed to analyze antimicrobial resistance patterns of the common pathogens occurring in the Federal Capital Territory and Lagos states of Nigeria. A retrospective analysis of microbiological culture results from five private and public laboratories between January and April 2016 in Lagos and the Federal Capital Territory (FCT). A total of 544 isolates were obtained from the five laboratories. Urine 137 (25.2%), was the most prominent specimen, followed by Stool 64 (11.7%). Out of 374 specimens that yielded growth after culture, gram-negative isolates were the most prevalent bacteria 169 (56%). There were significantly more samples from FCT and by gender (P=0.03) compared to Lagos (P=0.0003). There was no significant association between the type of bacteria isolate and location. The predominant gram-positive bacteria were *Staphylococcus aureus* 94 (70%), while gram-negative bacteria were *Klebsiella* species 67 (40%). Multi-drug resistance was observed in 202 (62%) of isolated urogenital pathogens. Maximum resistance was observed with commonly used first-line antimicrobials such as co-trimoxazole, tetracycline, second-generation cephalosporins -Cefotetan, and third generation Cephalosporins -Ceftriaxone. *Staphylococcus aureus* had the highest antimicrobial resistance expression (93.6%) within the gram positives, with multiple drug resistance to all the antibiotics. Our survey revealed a rising rate of antimicrobial resistance to commonly used antibiotics. We recommend the prudent use of antibiotics to limit the spread of antimicrobial resistance.

Keywords: Antimicrobial resistance; Nigeria; Antimicrobial susceptibility; Urogenital; *Staphylococcus aureus*

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INTRODUCTION

The advent of antibiotic-resistant strains has threatened to render existing treatments ineffective against many infectious diseases [1]. Globally, a projected 700,000 deaths will be attributed to antimicrobial resistance with an estimated 10 million deaths in 2050 [1]. The inappropriate use, overuse, sub-standard or counterfeit antibiotics have accelerated the emergence of antimicrobial resistance [2]. The emergence of multidrug resistance to several classes of antibacterial is a serious cause of concern. There is a notable increase in multi-drug resistant bacteria jeopardizing the effectiveness of antibiotic, unfortunately since the last major discovery of antibiotics in 1987, there have been too few drugs in development [2]. Multi-resistance strains of pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, and *Salmonella* Typhi have shown increasing resistance to first and second-generation antibiotics [3]. Urinary tract infections (UTIs) are considered the most common and most pathological health conditions acquired in hospitals and communities[4]. An estimated 50 percent of women have been infected with UTIs in their lifetimes than men. Treatment of UTIs varies according to age, sex, causative agent, and region of the urinary tract affected. However, drug resistance has been reported among bacteria causing UTIs [5]. Due to the growing emergence of antimicrobial resistance (AMR), the World Health Assembly adopted the Global Action Plan (GAP) on Antimicrobial Resistance in May 2015. There is increasing drug resistance reported for HIV programs and several pathogens in Africa. To ascertain the burden of AMR, one of the strategic objectives of GAP includes “to strengthen the knowledge and evidence base on AMR through surveillance and research” [6], .

Moreover, several initiatives have been set up to conduct studies on the burden of AMR such as ReACT and global antimicrobial resistance partnership, WHO’s Global action plan to develop and implement national action plans on AMR, and Africa CDC Anti-microbial Resistance Surveillance Network (AMRSNET) on AMR surveillance and control for Africa. As of 2016, Nigeria had no AMR surveillance network nor Antimicrobial Resistance Surveillance (AMRS) national data [7]. In line with the GAP, the country committed itself to establishing a national AMR surveillance system [8]. To understand the AMR situation in the country, the Federal ministries of health, agriculture, and environment conducted a situational analysis of antimicrobial use and resistance in certain geographic areas of the countries and found an alarming score of 70% - 100% resistance rate to some antibiotics [9]. Furthermore, UTIs’ accounted for a 3.4-88.5% prevalence rate with associated resistance organisms such as *Pseudomonas, E.coli, Klebsiella*, and *Staphylococcus* [8,10]. The Nigerian surveillance program is expected to monitor the susceptibility pattern of bacteria over time for improved decision-making. The surveillance program was set up with an initial network of ten clinical and public health laboratories that could conduct testing for antibiotic sensitivity. However, the quality management system was recognized as key in antimicrobial and sensitivity testing (AST), amongst several common challenges such as the lack of standardized operating procedures across the laboratories for susceptibility testing, quality assurance, data management, and reporting [11]. This lack of concordance has a huge implication on the data quality reported. To address this, Nigeria in conjunction with stakeholders developed a guideline for antimicrobial resistance laboratory surveillance in 2018 with the aim of providing a framework for AMR surveillance implementation and using standardized Standard Operating Procedure (SOP) within the participating network of labs. Nigeria like most countries has reported growing antimicrobial resistance requiring continuous monitoring of susceptibility patterns [12, 13, 14]. The exact prevalence of AMR in uro-vaginal microorganisms of public health importance in Nigeria is inadequate. This study was undertaken as part of a larger surveillance program to provide supporting data on antimicrobial resistance patterns of common pathogens with an emphasis on urogenital pathogens within Lagos state and the Federal Capital Territory of Nigeria.

METHODOLOGY

Study Area and Sites

This study was conducted in Federal Capital Territory and Lagos, Nigeria. Lagos and Federal Capital Territory are considered the economic hubs of the country with 20 and 6 local government and area councils respectively and according to the Health facility registry of the federal ministry of health, they both have about 2333 and 757 public and private registered health facilities respectively [15]. The laboratory departments of the public and private facilities receive patients from all socioeconomic strata.

Study Design and Population

The cross-sectional study using retrospective data was conducted in three health facilities (1 public health laboratory and 2 public health facilities) in Lagos and two health facilities (1 public health facility and 1 private health facility) in Abuja in March 2016. Microbiological culture logs were pulled from the facility registrar that is 3 months prior to the date of review specifically data from

January to March 2016. The facilities in Lagos and Abuja were purposively sampled after meeting the minimum criteria following the initial gap assessment.

Study Inclusion
The majority of facilities selected that met minimum criteria included those that perform microbial, culture, and sensitivity tests and had records of the following information such as age, sex, bacteria isolates, and culture results.

Data Collection Procedures
A structured checklist adapted from World Health Organization (WHO) laboratory assessment tool was used to assess over 40 facilities on several functionalities and capabilities ranging from availability of laboratory equipment, staffing, culture, and sensitivity testing among others [16]. A subset of these facilities (5) was purposively selected for AMRS assessment. After securing approval from the hospital administrations of purposively selected facilities, a structured data collection tool was used to collect AMR data from hospital records. Records of patients who presented for microscopy, culture, and sensitivity tests from the period of January to March 2016 were included in the study. Most of the laboratories sampled had variations in standardized operating procedures for antimicrobial susceptibility testing, data management, and reporting.

Facilities sampled in this study collected urine, pus, sputum, and vagina including high vagina swabs, urethral swabs, ear swabs, and semen. As of 2016, there were no national standard guidelines for collecting, processing, identifying, characterizing, and reporting antimicrobial resistance organisms in Nigeria. However, some of the laboratories performed similar microscopic identification as described by Cheesbrough in terms of media preparation and identification of isolates [17].

Culture and identification of isolates followed the standard operating procedures of the laboratory. Blood agar, MacConkey agar, and chocolate agar were used to culture and isolate the organisms. Gram staining and colony characteristics were used for presumptive identification. Enzymatic and biochemical confirmatory tests were performed on pure colonies following culture on nutrient agar for 24 hours at 37^0C.

Antibiotic Susceptibility Testing
Each microbiological bench in the laboratory's health facility visited performed lab susceptibility tests. The modified Kirby-Bauer disk diffusion method was employed to obtain antimicrobial susceptibility testing (AST) results according to the guidelines by Clinical Laboratory Standard Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST). This method involves aseptically inoculating bacteria samples on Mueller Hinton Agar using the spread method. Antibiotics discs are placed on the agar and incubated for 24 hrs at 35^OC. Zones of inhibition which are circular areas around the antibiotic discs are observed on the surface of the agar after incubation. A larger zone of inhibition indicates a stronger sensitivity while areas with no growth indicates total resistance. Laboratories used different antibiotic disks with their concentrations in micrograms produced by various manufacturers available in the market. In this study, multidrug-resistant isolates are classified as resistant to two or more antimicrobial classes. The classes of antibiotics studied were Cephalosporins (cefuroxime, ceftriaxone, cefotaxime), Penicillin (Amoxicillin, amoxicillin/clavulanic acid, ampicillin), Nitrofuran (Nitrofurantoin), Macrolides (Erythromycin), Aminoglycosides (gentamicin), Quinolones (Ofloxacin, ciprofloxacin), and tetracyclines (tetracycline).

Data Analysis
Unrelated sample data on age, sex, bacteria isolates, and culture results were pooled on the sample type, pathogen isolated, and the antimicrobial susceptibility results. Data were entered into a structured checklist using Excel and Descriptive analysis was conducted. Results were presented as absolute numbers and percentages. Chi square tests were used to compare the association between the type of bacteria isolates, specimen type and location at a significance level of 0.05. Antibiotic resistance pattern per listed antibiotic was calculated as the percentage of isolate resistant to specific antibiotic over the total number of resistant isolates.

Ethical Consideration
This is a retrospective study wherein de-identified patient-level data was collected and provided as part of a larger surveillance program. Hospital management of all health facilities that participated in this study also provided approval for data to be collected from facility registers.

Results
A total of 544 bacterial and fungal samples were collected during the study period. There were 102 males and 268 females and 174 (32.0%) missing records. Male to female ratio was 1:2.6. The majority, 71 (13.2%) of patients were between 31-45 years of age. However, about 177 (32.9%) of the age records were missing and 163 (29.9%) did not have age defined (specified as adult and child). Overall, age ranged from 5 months to 70

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years with a mean of 29.9 years (Table 1). Out of 544 samples collected, Urine 137 (25.2%), was the most prominent specimen followed by Stool 64 (11.7%). However, about 229 (42%) were missing records for the type of specimen collected. Of the 137 specimens collected from Urine, the most frequent pathogen isolated was *Staphylococcus aureus* 19 (13.87%), followed by Coliform sp 15 (10.95%) (Figure 1).

A total of 374 of the 544 specimens yielded growth after culture. Among those with positive bacteria growth, within the specified age groups, female 86% (16-49 years) were most affected. Moreover, of the 374 which yielded growth after culture*, Staphylococcus aureus* 94 (25%) was the most prominent isolated organism affecting both genders, with females 47 (60.3%) having a higher proportion than men. Moreover, there were more women reported with *Staphylococcus aureus* 44 (62%) and *E.coli* 23 (85%) in Abuja than in Lagos 4(66%). This is not significant (P=0.75 and P=0.52 respectively). Also, facilities in Abuja presented the highest proportion 70 (90%) of *Staphylococcus aureus* than Lagos followed by *E. coli* with majority of cases reported from facilities within Abuja 27 (77%). (Table 2). There were significantly more samples from FCT and by gender (P=0.03) compared to Lagos (P=0.0003). There was no significant association between the type of bacteria isolate and location. (Table 3).

Out of the samples that yielded growth (374), Gram-negative isolates were the most prevalent bacteria 169 (56%) and among the gram negatives, *Klebsiella* species 67 (40%) was the most detected while *Haemophilus influenza* 2 (1.2%) was the least isolate detected. Of the 134 (44%) Gram-positive isolates found, *Staphylococcus aureus* accounted for more than 94 (70%) of the isolates detected. (Table 4).

Staphylococcus aureus isolate was resistant to Ceftazidime (98%), and Ciprofloxacin (98%), while low resistance was observed for Nalidixic acid (32%). *Klebsiella* species and *E. coli* were most resistant to imipenem, ceftazidime, and ceftriaxone at 99% and 98%. The overall resistance rate was 97.6%, 98%, and 93.6% for *Klebsiella* species*, E.coli,* and *Staphylococcus aureus* respectively (Table 5).

On Multiple antibiotic resistance, *Klebsiella* species, *E. coli,* and *Staphylococcus aureus* were resistant to almost all the antibiotics tested. These isolates made up 202 (62%) of the total bacteria isolates. Overall, *Staphylococcus aureus* showed reduced sensitivity for all antibiotics tested (Table 6).

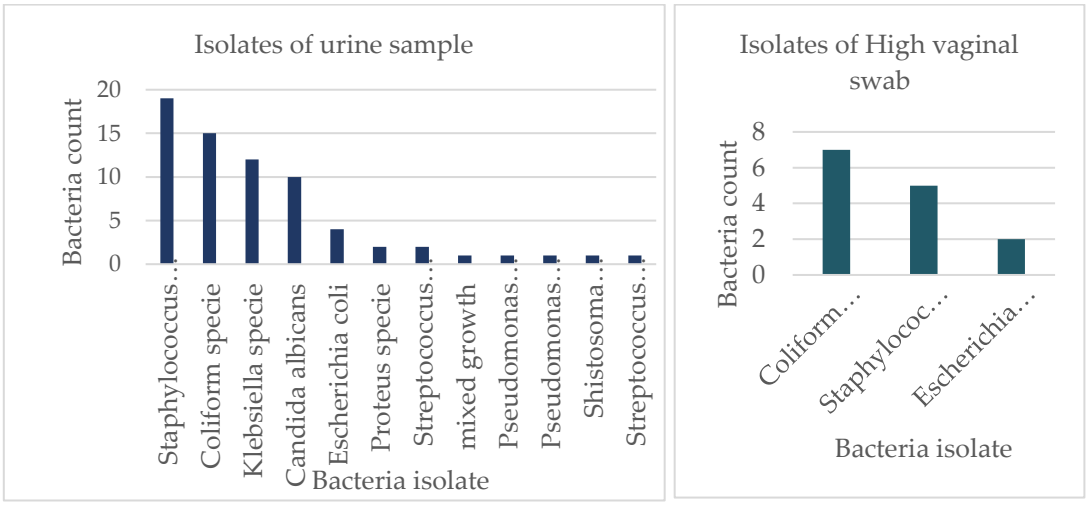

Figure 1: Distribution of isolates from urine and high vaginal samples

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Table 1: Sociodemographic variables

Variable	Frequency (%)
Gender	
Male	102 (18.8)
Female	268 (49.3)
Missing	174 (32.0)
Facility	149 (27.3)
Private facility	373 (68.5)
Public facility	22 (4)
Central Laboratory	
Clinical samples	60(11)
High vaginal swabs	137 (25.2)
Mid-stream urine	26 (4.8)
Sputum	64(11.8)
Stool	28(5.1)
Others	229(42.1)
Missing	
AGE	68(12.6)
1-15	40(7.5)
16-30	71(13.2)
31-45	20(3.7)
46-70	161(29.9)
Adults	177(32.9)
Missing	

Table 2: Association between some bacteria isolates and location

Bacteria Isolates	Frequency (n)	Location (n)		Test statistic	P Value
		Abuja	Lagos		
Klebsiella spp.	27	11	16	1.17	0.28
Females		9	10		
Males		2	6		
E. coli	35	27	8	0.42	0.52
Female		23	0		
Male		4	0		
S. aureus	77	70	7	0.1	0.75
Female		44	3		
Male		26	4		
Coliform Spp	18	16	2	0.31	0.58
Female		11	2		
Male		5	0		
Total	157	124	33		

Table 3: Buvariate analysis of bacteria isolates and gender by location

Variable	FCT (n)	Lagos (n)	Test statistics	P Value
Bacteria agents				
Coliform spp	24	2	163.76	5.411
Enterobacter spp	3	0		
Escherichia coli	29	12		
Haemophilus spp	1	1		
Klebsiella spp	11	56		
mixed growth	1	0		
Neisseria gonorrhoea	3	28		
Proteus spp	7	2		
Pseudomonas spp	3	4		
Salmonalla species	6	0		
Staphylococcus spp	122	12		
Total	210	117		
Specimen type				
Aspirate	3	0	32.70	0.0003*
Ear Swab	3	4		
Eye swab	1	0		
High vagina swab	25	35		
Semen	6	0		
Sputum	7	19		
Stool	49	15		
Urethral swab	3	0		
Urine	77	60		
Vaginal swab	0	1		
Wound swab	2	5		
Total	176	139		
Gender				
Male	160	106	4.37	0.036*
Female	74	29		
Total	234	135		

*Significant

Table 4: Distribution of bacteria isolates

Variable	Number (%)
Bacteria agents	
Escherichia coli	41(10.9)
Klebsiella species	67(17.9)
Neisseria gonorrhea	31(8.2)
Staphylococcus aureus	94(25.1)
Coliform species	26 (6.95)
Enterobacter species	3(0.8)
Haemophilus species	2(0.53)
Proteus species	9(2.4)
Pseudomonas species	7(1.8)
Salmonella species	6(1.60)
Streptococcus Species	40 (10.69)
Fungal agent	
Candida albica	43(11.47)

Table 5: Antibiogram showing the resistant profile of the bacteria isolates to different antibiotics

Organisms Bacteria	Number of Isolates (No)	% Resistant														
		Gentamycin	Ceftazidime	Amoxicillin	Ceftriaxone	Erythromycin	Cefuroxime	Amoxocillin_Clavulunate	Levofloxacin	Imipenem	Nalidixic acid	Norfloxacin	Nitrofurantoin	Tetracycline	Ciprofloxacin	Co-trimoxazole
Gram-Negative																
Klebsiella spp.	67	99	99	99	99	99	99	99	99	99	78	99	99	99	99	99
E. coli	41	98	98	98	98	98	98	98	98	98	-	98	98	98	98	98
Gram-Positive																
Staphylococcus aureus	93	98	98	98	98	98	98	98	98	98	32	98	98	98	98	98

Table 6: Antibiogram showing the susceptibility profile of the bacteria isolates to different antibiotics

Organisms Bacteria	Number of Isolates (No)	% Susceptible								
		Gentamycin	Ciprofloxacin	Amoxicillin+Clavuluate	Amoxicillin	Ofloxacin	Tetracycline	Nitrofurantoin	Ceftazidime	Nalidixic Acid
Klebsiella spp.	67	82	82	69	78	82	52	82	75	82
Staphylococcus aureus	93	32	32	32	32	32	32	32	33	32

DISCUSSION

Urovaginal pathogens have been responsible for the majority of infections, and this is exacerbated by the high cost of treatment. There is increasing antimicrobial resistance among urinary tract infections (UTIs). *E. coli* is documented as the predominant cause of symptomatic and non-symptomatic UTI. A higher proportion of females 23 (65%) than males were reported with *E. coli* in this study. The majority of *E. coli* cases were reported from facilities within Abuja 27 (77%) than in Lagos 8 (22%). The high prevalence of UTI among the female population in this study was similar to reports in Nigeria [18], including biological plausibility of occurrence due to decreased normal vagina flora and poor hygienic conditions. *Staphylococcus aureus* is the leading major cause of infection in health facilities. They are the most predominant gram-positive organisms consisting of about (25%) of bacterial isolates which is similar to what was reported in Lagos, Nigeria [12]. In this study, facilities in Abuja presented a significantly higher proportion 70 (90%) of *Staphylococcus aureus* than in Lagos 7 (0.09%). This is similar to a study conducted in Nigeria with 54.1% of *Staphylococcus aureus* identified in the North Central and 2.5% in the south-south region [19]. Overall, there was no significant association between the type of bacteria isolate and location in this study. Antibiotic Resistance to *S.aureus* in this study ranged from 32-98%. Similarly, other studies have reported multi-drug resistance of *S.aureus* [12,13,14,18]. Both genders have been almost equally affected by *Staphylococcus aureus* infection (49%) followed by *E. coli* (23%). This is

contrary to findings in Nigeria [13,18]. This study found low sensitivity such as *Klebsiella* spp. and *Staphylococcus aureus* to several antibiotics which is in agreement with several published reports in Pakistan [20] and Indonesia [21]. There was a high level of resistance to major antibiotics which could be a result of self-medication and antibiotic abuse [22].

Cephalosporins are used to treat a variety of bacterial infections and are also useful in the empirical treatment of urinary tract infections. The third-generation antimicrobial (ceftazidime and ceftriaxone) Cephalosporins are useful against a broad range of bacteria including gram-negative bacteria[23]. In this study, the majority of the gram-negative bacteria such as *E. coli* were resistant to the 3rd generation cephalosporins (ceftazidime and ceftriaxone). The reduced susceptibility of these gram negatives has been reported in other studies [24,25]. Multidrug resistance was observed at 97.6%, 98%, and 93.6% for *Klebsiella* species*, E. coli*, and *Staphylococcus aureus* respectively. The observed multi-drug resistance is higher than the result reported in Lagos (86.7%, 84.2%, 83%) [12]. This high level of resistance observed for penicillin, co-trimoxazole, and tetracycline can be attributed to the inexpensive nature of these drugs, relatively broad spectrum in nature, and on the list of essential medicines frequently in use in developing countries [22].

During this study, investigators found poor data management and reporting practices among the selected laboratories. For example, Missing data from facility records such as age 177 (32.9%) and sex 174 (32.0%) were observed. Reporting was mostly paper based. In addition, there were variations in standard laboratory practices regarding identification, culture, resistance, and sensitivity testing. For example, laboratories in Lagos had different reporting systems for culture and microscopy results making quality management a challenge. Moreover, some laboratories reported the unavailability of selective and differential media. Quality testing of reagents and media was rarely done. The use of different antibiotic discs at the time of this survey was also observed in the majority of laboratories sampled. These challenges highlighted were acknowledged by the Nigerian Center for Disease Control in 2018 as an issue requiring a structured approach through the development of a national laboratory guideline to guide processes for isolation, identification, and AMR testing [10,11]. Regarding antimicrobial susceptibility protocol, there are GLASS recommendations for antibiotics testing and recommended antibacterial agents. Although allowance has been provided for testing according to local prescribing practices, this study found a lot of antibiotics testing conducted for bacterial isolates outside the GLASS recommendations. We recommend appropriate SOP be developed and used according to the most current guidelines of CSLI or EUCAST.

STUDY LIMITATION

Out of the facilities (40) that met the minimum criteria for microscopic culture and sensitivity testing capacity, only a subset of the facilities (5) provided permission to conduct this study. Quality assurance in laboratory procedures from specimen receipt, handling, testing, analysis, and reporting needs to be reliable to meet the appropriate regulatory requirement. In this study, we observed that several antibiotic testing and sensitivity (ATS) laboratory processes and procedures will need to be strengthened to assure the safety and effectiveness of the tests provided.

CONCLUSIONS

The most prevalent bacteria isolated in this study is *Staphylococcus aureus* from urine specimens. Although Gram-negative bacteria were the most isolated in terms of number, the *Staphylococcus aureus* –a Gram-positive bacterium were the most predominant isolate. Several bacterial isolates showed high resistance to first-line antimicrobials like amoxicillin and second-line antimicrobials like fluoroquinolones indicating the need for regular monitoring. Moreover, it is imperative that hospitals use microscopic culture and sensitivity (MCS) data to procure antibiotics. Facilities should consider the development of antibiograms for the clinical management process. Routine MCS testing can be valuable for AMR surveillance. There is an urgent need for Improvement in Microscopic sensitivity and culture sensitivity documentation through continuing education and congruent record-keeping practices across laboratories.

ABBREVIATIONS

AMR - Antimicrobial Resistance
AMRSNET - Anti-microbial Resistance Surveillance Network
AST - Antimicrobial and sensitivity testing
CLSI - Clinical and Laboratory Standards Institute
EUCAST - European Committee for Antimicrobial Susceptibility Testing
FCT - Federal Capital Territory
GAP - Global Action Plan
GLASS - Global Antimicrobial Resistance and Use Surveillance System
HIV - Human Immunodeficiency Virus
ReACT - Action on Antibiotic Resistance
SOP - Standard Operating Procedure
UTI - Urinary Tract Infections
WHO - World Health Organization

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
HA, MN, BG, and TO participated in the design of the experiment, methodology, validation, and Analysis; KN, AI, JS, RI, WU, EM, AE, and SO participated in the validation, formal analysis, and investigation; HA AI, JS, KN, WU prepared the original draft; MN, RI, BG, SA, OO, EM, AE, SO review and edited the manuscript and visualization, MN, and BG supervised and provided overall project administration.

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Susceptibility Patterns of Multiple Antibiotic-Resistant Bacteria from Wound and Urine Samples to the Extract of *Spondias mombin* (Linn)

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ABSTRACT

This study evaluates the antibacterial activity of *Spondias mombin* L. against multiple antibiotic-resistant bacteria isolated from wound and urine samples of patients attending five (5) selected hospitals in Akure, Ondo State, Nigeria. A total of 313 bacterial isolates were recovered from 353 samples of wound and urine using standard bacteriological procedures, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most predominant in urine and wound samples, respectively. The methanolic extract of *S. mombin* was the most effective against wound isolates, while the aqueous extract was the most effective against urine isolates. The results showed that the methanol extract of *S. mombin* had a zone of inhibition of 24.00±0.00, 30.67±0.33 and 19.33±0.33 mm respectively, against *S. aureus, S. epidermidis* and *P. aeruginosa* at 100 mg/ml. The aqueous extract had a zone of inhibition of 24.67±0.33, 27.33±0.33, 18.67±0.33, 24.67±0.33, 23.67±0.33, 21.33±0.33 and 21.67±0.33 mm against *Escherichia coli*, *Klebsiella pneumoniae, P. aeruginosa, Proteus mirabilis, S. aureus, S. saprophyticus* and *Trichomonas vaginalis* respectively, at 100 mg/ml. The phytochemical constituents of the extracts include alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins, steroids and tannins. These compounds may be responsible for the antibacterial activity of *S. mombin* against the multiple antibiotic-resistant bacterial isolates. The findings of this study demonstrate the potential of *S. mombin* as an alternative treatment for multiple antibiotic-resistant bacteria from wound and urine.

Keywords: Antibiotic resistance; *Spondias mombin*; Bio-active compounds.

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INTRODUCTION

Antibiotic resistance is a major global health crisis that threatens the effective treatment of infectious diseases. When bacteria acquire the ability to withstand antimicrobial medications that are meant to kill them, antibiotic resistance arises. This resistance can lead to the emergence of "superbugs" that are difficult or impossible to treat with currently available antibiotics [1]. The overuse and inappropriate use of antibiotics are major drivers of the emergence and spread of antibiotic resistance [2]. In addition, the widespread use of antibiotics in agriculture and aquaculture has also contributed to the development of antibiotic resistance in bacteria [3]. The consequences of antibiotic resistance are significant and potentially catastrophic. Antibiotic resistance causes 700,000 global deaths annually, with rising numbers expected unless urgent action is taken. Economic costs include longer hospital stays, expensive treatments, and decreased productivity [4]. In the face of this crisis, there is a pressing need to identify alternative antimicrobial agents that can effectively treat multiple drug-resistant infections.

Plant-derived compounds have been used for centuries in traditional medicine due to their antimicrobial properties [5]. Plant-based antimicrobials offer a promising alternative to synthetic ones, potentially reducing antibiotic resistance, attracting increasing scientific interest in addressing the problem [6]. One such plant is *Spondias mombin*, commonly known as the yellow mombin or hog plum in English, known as Iyeye in Yoruba, Ijikara (Igbo), and Tsardar masar (Hausa). *S. mombin,* originating from Central and South America, is a fruit-bearing tree that thrives in tropical regions. It has a long history of use in traditional medicine for the treatment of a variety of infections [7]. The fruit, leaves and bark of the tree have been used to treat a range of conditions, including stomachache, diarrhea, wound, fever and urinary tract infections [7].

Recent studies highlight the potential of *S. mombin* as an antimicrobial agent due to its phytochemicals, particularly saponins, which exhibit potent antimicrobial activity against various bacteria and fungi [7, 8]. *S. mombin* fruit, known for its antimicrobial and anti-inflammatory properties, has hypoglycemic effects in animal studies, suggesting potential as a natural diabetes treatment [9]. *S. mombin* leaf extracts have also been shown to have hypolipidemic effects in animals, meaning that they may help to lower levels of cholesterol and other lipids in the blood [9]. Despite the various medicinal properties of *S. mombin*, more research is needed to fully understand its therapeutic potential and to identify the active components responsible for its effects.

S. mombin has a long history of traditional use in various cultures. The fruit of the tree is edible and is often consumed fresh or processed into jams, jellies, and beverages [10]. In traditional medicine, different parts of the plant, including the leaves, bark and fruits, have been used for their medicinal properties. It has been employed to treat various conditions such as diarrhea, dysentery, wound healing and fever [11].

The chemical composition of *S. mombin* has been studied, revealing the presence of bioactive compounds that contribute to its medicinal properties. Phytochemical analysis of the plant revealed various constituents, including phenolic compounds, flavonoids, tannins, alkaloids, saponins and terpenoids [11, 12]. These compounds are known for their potential biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer properties.

These findings suggest that *S. mombin* may possess broad-spectrum antimicrobial properties, making it a promising candidate for further exploration and development as a natural antimicrobial agent. The broad-spectrum antimicrobial activity of *S. mombin* extracts indicates their potential for addressing the challenges posed by antibiotic-resistant bacteria. Saha conducted a phytochemical screening of *S. mombin* leaf extracts and reported the presence of flavonoids, tannins, alkaloids, saponins, and phenolic compounds [13]. Similarly, Bossou et al. [12] performed a comprehensive phytochemical analysis of *S. mombin* and identified various bioactive components, including phenolic acids, flavonoids, tannins, terpenoids, and alkaloids. These studies provide valuable information about the chemical constituents present in *S. mombin* extracts, which may contribute to their antimicrobial properties.

Thus, the main objective of this study was to evaluate the antibacterial activity of *S. mombin* (L.) against multiple antibiotic-resistant bacteria isolated from wound and urine samples of patients attending five selected hospitals in Akure, Nigeria.

METHODOLOGY

Study Population

Seventy-one wound samples and 282 urine samples were collected from patients in five Akure hospitals, including diabetic, accident, burns, and postoperative wounds, and from patients with and without urinary tract infection history.

Wound and Urine Sample Collection

Wounds were cleansed with sterile normal saline, and wound swabs were collected from all participants using sterile moistened cotton swabs in an aseptic manner. The swabs were then placed in an icepack container and transported to the laboratory [14]. Urine samples were collected using sterile universal bottles and transferred to the research laboratory for further processing [15].

Isolation and Identification Method
Wound swab samples were immediately placed in Mueller Hinton broth, followed by streaking on Mannitol Salt Agar (MSA), Nutrient Agar, and Blood agar plates. The plates were incubated aerobically at 37°C for 24 hours. Presumptive identification of bacteria was based on their cultural characteristics on each agar plate. Representative colonies were sub-cultured on MSA plates and Nutrient agar, and further incubated at 37°C for 24 hours. The distinct, well-isolated colonies were then studied for their cultural and morphological characteristics. Gram staining and biochemical tests were conducted to confirm the identification of the isolates [16].
For urine samples, 1 µl of urine was spread quantitatively on MacConkey agar, and CLED agar, and incubated aerobically at 35°C for 24 hours. Similar procedures for presumptive identification, sub-culturing, incubation, and confirmation were followed for the urine samples [15]. Preliminary characterization of the isolates was conducted using Gram staining, morphological examination, cultural characteristics and biochemical tests according to Shoaib et al*.* [17].

Antibiotics Susceptibility Testing
The susceptibility of the isolates to antibiotics was determined using the disk diffusion method as described by Cheesbrough [18]. Gram-positive isolates were tested against eight commercially available antibiotics CAZ(30µg), CRX(30µg), GEN(10µg), CTR(30µg), ERY(5µg), CXC(5µg), OFL(5µg), AUG(30µg), while Gram-negative isolates were tested against eight different antibiotics CAZ(30µg), CRX(30µg), GEN(10µg), CXM(10µg), OFL(5µg), AUG(30µg), NIT(30µg), CPR(10µg). The zones of inhibition were compared with CLSI guidelines.

In Vitro Antibacterial Studies
The leaves were obtained from a farm in Akungba Akoko and confirmed at the department of Crop, Soil and Pest Management at Federal University of Technology, Akure. The leaves were dried and ground into coarse powder using a blender. Extraction and standardization of plant extracts were carried out following established procedures as described by Okiti and Osuntokun [19]. The agar well diffusion method was employed for the antibacterial studies. The dried plant extract was reconstituted with sterile distilled water and ethyl acetate to obtain different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Bacterial strains were cultured and spread on Mueller-Hinton agar plates, and wells were made in the agar. The plant extracts were introduced into the wells at different concentrations, and ciprotab (2 mg/ml) was used as control. The plates were incubated at 37°C and the zones of inhibition were measured to determine the antibacterial activity of the plant extract [20].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC of the extracts against the test organisms was determined using the broth dilution method described by Rankin and Coyle [21]. Serial dilutions of the extracts were prepared to obtain extract concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, and their inhibitory concentrations were recorded. The MBC was determined using the method established by the Mesbah et al. [22]. Samples that did not show visible growth after incubation were streaked on Nutrient Agar plates to determine the minimum concentration of the extract required to kill the organisms. The lowest concentration indicating a bactericidal effect was recorded as the MBC.
The qualitative phytochemical tests were carried out using the method described by the following authors: Alkaloid test [23], Anthraquinone test [24, 25], Cardiac Glycosides test [23, 25]. Flavonoid test [23, 24, 26], Phenol test [24], Saponin test [23, 27], Steroid test [23], Tannin test [24, 27].

Statistical Analysis: Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 20.0 version. Differences were considered significant at $P \leq 0.05$.

RESULTS
A total of 353 samples were analyzed for the presence of multiple antibiotic-resistant bacteria. 59 bacterial isolates were recovered from 67 wound samples collected. The predominant bacteria isolated from the infected wounds were *S. aureus* 39 (66.10%) followed by *P. aeruginosa* 15 (25.42%), and *S. epidermidis* 5 (8.47%). A total of 254 bacterial isolates were recovered from 282 urine samples. The predominant bacteria isolated from the urine samples were *P. aeruginosa* 84 (33.07%), followed by *S. aureus* 71 (27.95%), *S. saprophyticus* 41 (16.14%), *E. coli* 25 (9.84%), *T. vaginalis* 19 (7.48%), *K. pneumonia* 9 (3.54%), and *Proteus* sp. 5 (1.97%).

Table 1: Antibiotic sensitivity profile of bacteria isolated from wound

Isolates	Antibiotics to which isolates were resistant	MAR Index
S. aureus	CAZ, CRX, GEN, CTR, ERY, CXC, OFL, AUG	0.8
P. aeruginosa	CAZ, CRX, GEN, CXM, OFL, AUG, NIT, CPR	0.8
P. aeruginosa	CAZ, CRX, CXM, OFL, AUG, CPR	0.6
S. aureus	CAZ, CRX, CXC, AUG	0.4
S. epidermidis	-	0
S. epidermidis	CAZ	0.1
S. aureus	GEN, OFL	0.2

Key: CAZ – Ceftazidime (30µg), CRX - Cefuroxime (30µg), GEN - Gentamycin (10µg), CXM – Cefixime (10µg), OFL – Ofloxacin (5µg), AUG – Augmentin (30µg), NIT – Nitrofurantoin (30µg), CPR – Cefpirome (10µg).

Table 2: Antibiotic sensitivity profile of bacteria isolated from urine

Isolates	Antibiotics to which isolates were resistant	MAR Index
K. pneumoniae	-	0
P. mirabilis	CRX, OFL, AUG, CPR	0.4
E. coli	AUG	0.1
P. aeruginosa	CAZ, CRX, GEN, CXM, AUG, NIT	0.6
S. saprophyticus	CAZ, CRX, CTR, CXC, AUG	0.5
K. pneumoniae	CAZ, CRX, GEN, CTR, ERY, CXC, AUG	0.7
S. aureus	CAZ, CRX, ERY, CXC, AUG	0.5
P. mirabilis	AUG	0.1
S. aureus	CAZ, CRX, CTR, CXC, AUG	0.5

Key: CAZ – Ceftazidime (30µg), CRX - Cefuroxime (30µg), GEN - Gentamycin (10µg), CXM – Cefixime (10µg), OFL – Ofloxacin (5µg), AUG – Augmentin (30µg), NIT – Nitrofurantoin (30µg), CPR – Cefpirome (10µg).

Table 1 shows the antibiotic susceptibility patterns of several antibiotic-resistant bacteria obtained from wound samples. The MAR index was computed by dividing the number of antibiotics the bacteria were resistant to by the total number of antibiotics tested, and then multiplied by 100. The total number of antibiotics utilized per disc was 8. *S. aureus* and *P. aeruginosa* exhibited the highest MAR indices of 0.8 each, while a strain of *S. epidermidis* displayed susceptibility to all antibiotics, resulting in a MAR index of 0.

Table 2 presents the antibiotic susceptibility profiles of bacteria isolated from urine samples. *K. pneumoniae* had the highest MAR index at 0.7, followed by *P. aeruginosa* with a MAR index of 0.6. On the other hand, *P. mirabilis* exhibited a MAR index of 0.1.

Plates 1 and 2 show the sensitivity pattern of *S. aureus* against antibiotics for Gram-positive bacteria. It was observed that *S. aureus* was more susceptible to ofloxacin compared to other antibiotics.

Plates 3 and 4 show the sensitivity pattern of *P. aeruginosa* and *K. pneumoniae* against antibiotics for Gram-negative bacteria. *K. pneumoniae* was susceptible to gentamicin, ofloxacin, and cefpirome, while *P. aeruginosa* exhibited high resistance to the tested antibiotics. However, gentamicin demonstrated some level of activity against *P. aeruginosa.*

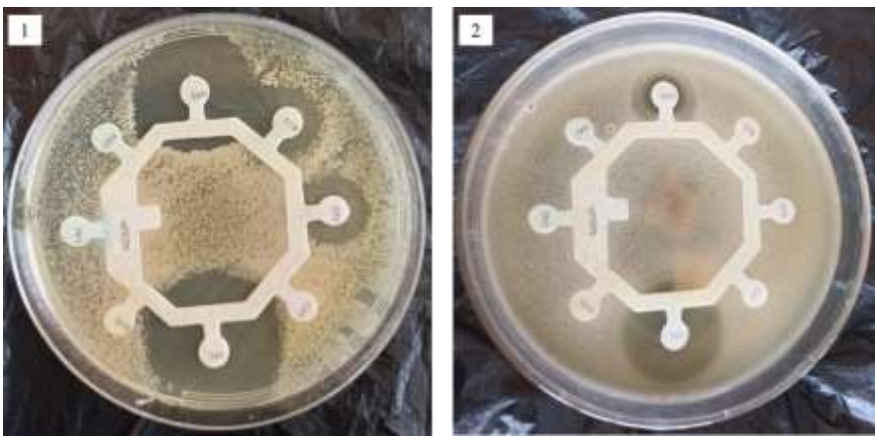

Plate 1 and 2: Antibiotic sensitivity pattern of *S. aureus*

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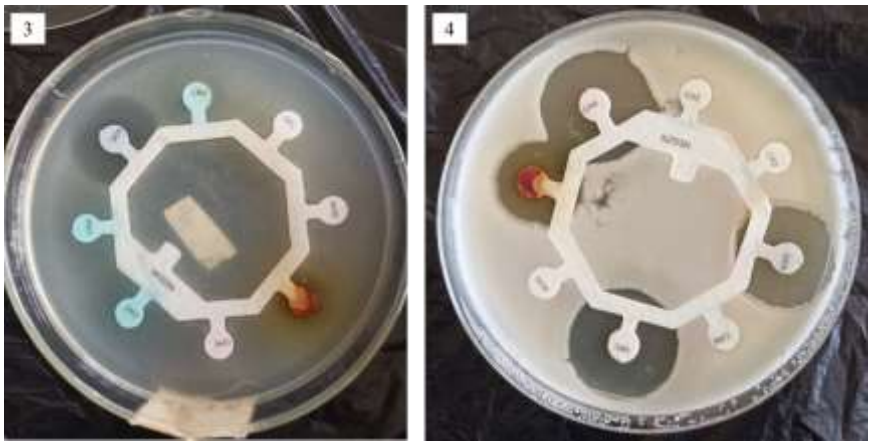

Plate 3 and 4: Antibiotic sensitivity pattern of *P. aeruginosa* and *K. pneumoniae*

Figures 1 to 3 show the susceptibility patterns of key isolates; *S.aureus* and *P. aeruginosa*, from wound and urine samples. In Figure 1, *S. aureus* was susceptible to gentamicin and ofloxacin while displaying resistance to the other antibiotics. Figure 2 illustrates the sensitivity pattern of *P. aeruginosa*, showing complete resistance to ceftazidime, cefuroxime, cefixime, augmentin, and cefpirome. Additionally, as shown in Figure 3, *P. aeruginosa* was also resistant to nitrofurantoin.

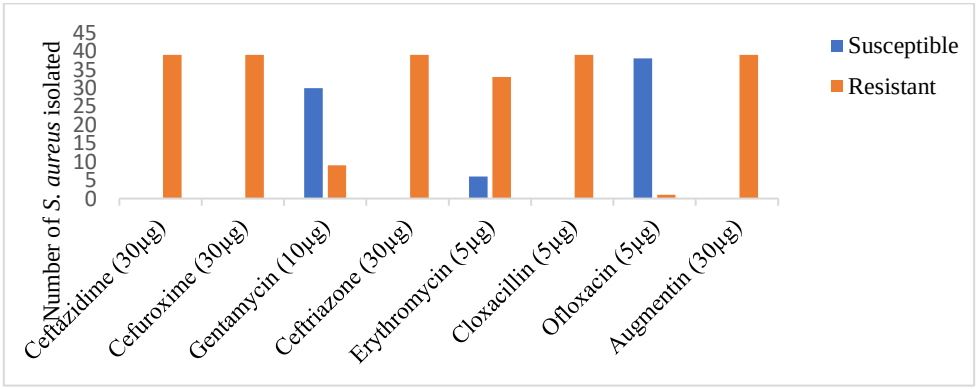

Figure 1: Susceptibility pattern of *S. aureus* isolated from wounds to conventional antibiotics

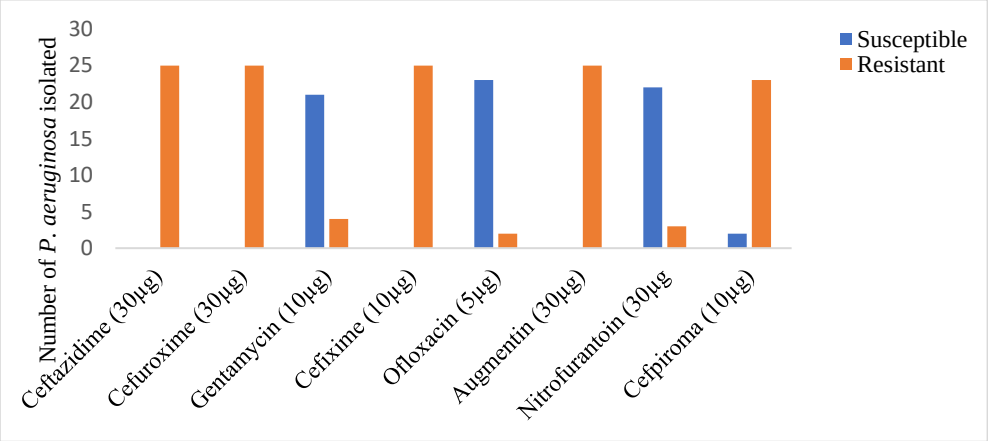

Figure 2: Susceptibility pattern of *P. aeruginosa* isolated from wounds to conventional antibiotics

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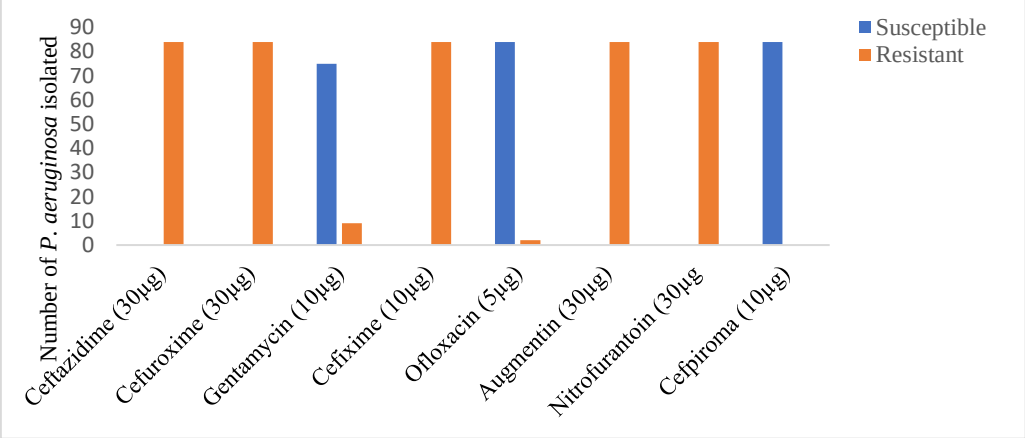

Figure 3: Susceptibility pattern of *P. aeruginosa* isolated from urine to conventional antibiotics.

Table 3-5 present the antibacterial activity of three different extracts—aqueous, methanol, and n-hexane against isolates from wound samples at various concentrations. The results are compared to positive and negative controls, where CiproTab (2 mg/m/) serves as the positive control, and water, methanol, or n-hexane are the negative controls, depending on the extract used.

In Table 3, both *S. aureus* and *S. epidermidis* showed significant antibacterial activity across all concentrations, with larger zones of inhibition at higher concentrations (100 mg/ml). *P. aeruginosa* displayed some sensitivity at higher concentrations but was completely resistant at the lowest concentration (12.5 mg/ml).

For the methanol extract in Table 4, *S. aureus* and *S. epidermidis* exhibited antibacterial activity, which decreased with decreasing concentrations, while *P. aeruginosa* showed moderate resistance, with no activity observed at the lowest concentration.

In Table 5, n-hexane extract was less effective overall, showing reduced antibacterial activity compared to the other extracts. *S. aureus* had no inhibition at lower concentrations, while *S. epidermidis* and *P. aeruginosa* showed declining activity with decreasing concentrations, with *P. aeruginosa* showing some resistance.

Table 3: Antibacterial activity of aqueous extract of on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C (+)	C(-)
S. aureus	24.67±0.33^{d}	21.00±0.00^{c}	17.33±0.33^{b}	11.33±0.67^{a}	23.00±1.00^{cd}	-
S. epidermidis	28.33±0.33^{d}	24.67±0.33^{c}	20.67±0.33^{b}	14.67±0.33^{a}	26.00±2.00^{cd}	-
P. aeruginosa	18.67±0.33^{d}	14.67±0.33^{c}	10.33±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Water.

Table 4: Antibacterial activity of methanol extract on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
S. aureus	24.00±0.00^{d}	21.00±0.58^{c}	18.33±0.67^{b}	14.67±0.33^{a}	23.00±1.00^{cd}	-
S. epidermidis	30.67±0.33^{d}	26.67±0.33^{c}	22.67±0.33^{b}	18.00±0.00^{a}	26.00±2.00^{c}	-
P. aeruginosa	19.33±0.33^{d}	16.00±0.00^{c}	13.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Methanol.

Table 5: Antibacterial activity of n-Hexane extract on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
S. aureus	18.67±0.33^{d}	14.00±0.58^{c}	10.33±0.33^{b}	0.00±0.33^{a}	23.00±1.00^{d}	-
S. epidermidis	26.00±0.58^{d}	23.67±0.33^{c}	18.33±0.33^{b}	11.67±0.00^{a}	26.00±2.00^{d}	-
P. aeruginosa	16.67±0.33^{d}	14.67±0.33^{c}	11.67±0.33^{b}	8.33±0.33^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control – n-Hexane.

Tables 6 – 8 show the antibacterial activity of aqueous, methanol, and n-hexane extracts against isolates from urine samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *S. saprophyticus,* and *T. vaginalis*) at concentrations of 100, 50, 25, and 12.5 mg/ml compared with positive and negative controls.

In Table 6, the aqueous extract showed notable antibacterial activity against all isolates, with larger inhibition zones at higher concentrations. *K. pneumoniae* and *P. mirabilis* were particularly susceptible, while *P. aeruginosa* displayed less sensitivity at lower concentrations, showing no activity at 12.5 mg/ml. In Table 7, similar to the aqueous extract, methanol extract displayed antibacterial activity across most isolates. The efficacy decreased as the concentration reduced, with *P. aeruginosa* and *P. mirabilis* exhibiting resistance at lower concentrations (12.5 mg/ml).

n-Hexane extract (Table 8) showed lower antibacterial activity compared to the aqueous and methanol extracts. *E. coli* and *K. pneumoniae* were inhibited at higher concentrations, while *P. aeruginosa* and *T. vaginalis* demonstrated reduced sensitivity, and several isolates including S*. aureus* and *S. saprophyticus*) showed no inhibition at the lowest concentrations.

Table 6: Antibacterial activity of aqueous extract on isolates from urine samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	24.67±0.33^{d}	21.00±0.00^{c}	16.00±0.00^{b}	10.33±0.33^{a}	29.00±1.00^{d}	-
K. pneumoniae	27.33±0.33^{d}	24.33±0.33^{c}	18.33±0.33^{b}	12.67±0.33^{a}	31.00±1.00^{d}	-
P. aeruginosa	18.67±0.33^{d}	15.67±0.33^{c}	11.67±0.33^{b}	0.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	24.67±0.33^{d}	22.33±0.33^{c}	18.00±0.00^{b}	11.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	23.67±0.33^{d}	15.67±0.33^{c}	10.67±0.33^{b}	9.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	21.33±0.33^{d}	17.67±0.33^{c}	11.67±0.33^{b}	9.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	21.67±0.33^{d}	16.67±0.33^{c}	12.33±0.33^{b}	9.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab – 2 mg/ml), C (-): Negative Control - Water.

Table 7: Antibacterial activity of methanol extract on isolates from urine samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	22.00±0.00^{d}	18.00±0.00^{c}	13.00±0.00^{b}	7.00±0.00^{a}	29.00±1.00^{d}	-
K. pneumoniae	25.00±0.00^{d}	21.00±0.00^{c}	18.00±0.00^{b}	14.00±0.00^{a}	31.00±1.00^{d}	-
P. aeruginosa	17.00±0.00^{d}	15.00±0.00^{c}	12.00±0.00^{b}	0.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	20.00±0.00^{d}	17.67±0.33^{c}	13.00±0.00^{b}	0.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	20.67±0.33^{d}	18.00±0.00^{c}	14.67±0.33^{b}	11.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	18.33±0.33^{d}	15.67±0.33^{c}	13.00±0.00^{b}	9.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	20.33±0.33^{d}	15.67±0.33^{c}	13.00±0.00^{b}	9.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Methanol.

Table 8: Antibacterial activity of n-Hexane extract on isolates from urine samples.

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	18.00±0.00^{d}	15.00±0.00^{c}	13.00±0.00^{b}	7.00±0.00^{a}	29.00±1.00^{d}	-
K. pneumoniae	19.00±0.00^{d}	15.00±0.00^{c}	11.00±0.00^{b}	9.00±0.00^{a}	31.00±1.00^{d}	-
P. aeruginosa	17.00±0.00^{d}	14.00±0.00^{c}	12.00±0.00^{b}	8.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	19.00±0.00^{d}	17.00±0.00^{c}	14.00±0.00^{b}	9.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	16.00±0.00^{d}	14.00±0.00^{c}	11.00±0.33^{b}	0.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	18.00±0.00^{d}	16.00±0.00^{c}	13.00±0.00^{b}	0.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	15.00±0.00^{d}	11.00±0.00^{c}	9.00±0.00^{b}	0.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab – 2 mg/ml), C (-): Negative Control - n-Hexane.

Table 9 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the plant extracts against the isolates from wound samples. For *P. aeruginosa*, all the extracts displayed a MIC value of 50mg/ml and an MBC value of 100mg/ml. *S. epidermidis* exhibited a MIC value of 12.5mg/ml and an MBC value of 25mg/ml. In the case of *S. aureus*, both the aqueous and methanol extracts had a MIC value of 25mg/ml, while the n-hexane extract had a higher MIC value of 50 mg/ml. Additionally, the MBC value for the n-hexane extract against *S. aureus* was determined to be 100 mg/ml.

Table 10 displays the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the plant extracts against the isolates derived from urine samples. The isolates, including *E. coli, S. aureus, S. saprophyticus, T. vaginalis, K. pneumoniae, P. mirabilis* and *P. aeruginosa*, exhibited MIC and MBC values ranging from 25 mg/ml to 100 mg/ml. Specifically, *P. aeruginosa* displayed MIC values of 25 mg/ml, 50 mg/ml and 25 mg/ml for the aqueous, n-hexane and methanol extracts.

Table 11 highlights the presence (+) or absence (-) of various phytochemicals in aqueous, methanol, and n-hexane extracts of *S. mombin*. Alkaloids, Flavonoids, Phenols, and Tannins were Present in all extracts. Anthraquinones and Cardiac Glycosides were detected only in water and methanol extracts. Saponins were found in water and methanol extracts but absent in n-hexane. Steroids were present in water and n-hexane extracts, but absent in methanol.

Table 9: MIC and MBC values of isolates from wound samples

MIC Isolates (mg/ml)	Aqueous	N-hexane	Methanol	MBC Aqueous	N-hexane	Methanol
S. aureus	25	50	25	50	100	50
S. epidermidis	12.5	12.5	12.5	25	25	25
P. aeruginosa	50	50	50	100	100	100

Table 10: MIC and MBC values of isolate from urine samples

MIC Isolates (mg/ml)	Aqueous	N-hexane	Methanol	MBC Aqueous	N-hexane	Methanol
E. coli	50	50	50	100	100	100
K. pneumoniae	25	25	25	50	50	50
P. aeruginosa	25	50	25	50	100	50
P. mirabilis	25	25	25	50	50	50
S. aureus	50	50	50	100	100	100
S. saprophyticus	25	25	25	50	50	50
T. vaginalis	50	50	50	100	100	100

Table 11: Qualitative Phytochemical Profile of *S. mombin*

Phytochemicals	Water	Methanol	n-Hexane
Alkaloids	+	+	+
Anthraquinones	+	-	-
Cardiac Glycosides	+	+	-
Flavonoids	+	+	+
Phenols	+	+	+
Saponins	+	+	-
Steroids	+	-	+
Tannins	+	+	+

Key- +:Positive, - :Negative

DISCUSSION

This study investigated the susceptibility patterns of multiple antibiotic-resistant bacteria isolated from wound and urine samples to the extract of *S. mombin*. The findings revealed a high prevalence of multiple antibiotic-resistant bacteria in both sample types, with *P. aeruginosa* being the most commonly isolated bacterium in urine samples and *S. aureus* being the most commonly isolated bacterium in wound samples. These results align with previous research indicating the prominence of *S. aureus* and *P. aeruginosa* as common antibiotic-resistant bacteria. Liu and Qin [28] reported similar findings, identifying these two bacteria as among the top five antibiotic-resistant pathogens in recent years, reflecting their well-known association with nosocomial infections and their ability to develop antibiotic resistance. The high prevalence of antibiotic-resistant bacteria in clinical samples highlights the need for effective antimicrobial strategies. The identification of other bacterial species in wound and urine infections highlights the complexity of the microbial landscape, guiding the development of appropriate therapeutic approaches.

Antibiotic resistance profiles of isolates revealed that *S. aureus* and *P. aeruginosa* showed the highest MAR indices of 0.8, indicating resistance to 80% of tested antibiotics. A strain of *S. epidermidis* displayed susceptibility to all antibiotics, indicating a generally less resistant bacterial strain compared to other *Staphylococcus* species. These results align with previous studies that have identified *S. aureus* and *P. aeruginosa* as major contributors to antibiotic resistance [28]. Table 2 provides further insights into the antibiotic susceptibility profiles of bacteria isolated from urine samples. The results indicate that *K. pneumoniae* exhibited the highest MAR index of 0.7, reflecting resistance to 70% of the antibiotics tested. This finding is in line with previous research that has highlighted the high prevalence of multidrug-resistant *K. pneumoniae* strains [29]. Similarly, *P. aeruginosa* showed resistance to 60% and *Proteus mirabilis* had a lower resistance index, this finding does not agree with existing studies that have

recognized *Proteus* mirabilis as a high commonly encountered multidrug-resistant pathogen [30].

The aqueous extract of *S. mombin* showed susceptibility against *S. aureus, S. epidermidis*, and *P. aeruginosa,* with zone diameters greater than those produced by ciprotab (24.67±0.33d, 28.33±0.33d), suggesting that the aqueous extract of *S. mombin* may possess stronger antimicrobial activity against these bacterial isolates. The methanolic extract also showed susceptibility to these bacteria, with larger zones of inhibition than the positive control. The n-Hexane extract showed varying degrees of effectiveness against antibiotic-resistant isolates from wound samples, with zone diameters varying from 0.00±0.33a to 8.33±0.33a.

The observed antimicrobial activity of the extracts of *S. mombin* in this study aligns with existing research on the plant's medicinal properties. Previous studies have reported the presence of various bioactive compounds in *S. mombin*, including alkaloids, flavonoids, and phenols, which have been associated with antimicrobial activity [31]. Additionally, the effectiveness of *S. mombin* against antibiotic-resistant bacteria corroborates findings from other studies that have investigated the antimicrobial potential of plant extracts against multidrug-resistant pathogens [32].

Results in this study highlight the antibacterial effectiveness of different extracts of *S. mombin* against multiple antibiotic-resistant bacterial isolates obtained from urine samples. These findings contribute to the existing research on the antimicrobial properties of *S. mombin* and support its potential use as an alternative therapeutic option for treating antibiotic-resistant urinary tract infections [12].

The study reveals the antibacterial properties of *S. mombin* extracts against various antibiotic-resistant bacteria from urine samples. The aqueous extract showed the highest inhibition zone against *K. pneumoniae* (27.33±0.33d), while the methanolic extract showed the highest inhibition zone against all isolates, with zone diameters ranging from 17.00±0.00 to 25.00±0.00 at a concentration of 100 mg/ml. The n-Hexane extract also showed the highest inhibition zone against all isolates, with smaller zones of inhibition than the positive control. These findings support the potential of *S. mombin* as an alternative therapeutic option for treating antibiotic-resistant urinary tract infections. The results support the potential of *S. mombin* as a potential therapeutic option.

The findings of this study are consistent with previous research on the antimicrobial properties of *S. mombin* extracts. Other studies have reported the presence of bioactive compounds in *S. mombin*, such as flavonoids, tannins, and alkaloids, which are known for their antimicrobial activities [32]. Furthermore, studies investigating the antimicrobial potential of *S. mombin* extracts against antibiotic-resistant bacteria have shown promising results [7]. These findings support the notion that *S. mombin* extracts may serve as valuable resources for the development of new therapeutic agents against antibiotic-resistant urinary tract infections.

The higher effectiveness of the aqueous extract compared to the methanol and n-hexane extracts suggests that the water-soluble components of *S. mombin* may play a significant role in its antimicrobial activity. This finding aligns with previous studies that have reported the antimicrobial properties of aqueous extracts of *S. mombin* against various bacterial strains [33]. The presence of bioactive compounds such as alkaloids, flavonoids, phenols, and tannins in the aqueous extract, as identified in previous phytochemical analyses [13], may contribute to its observed antibacterial activity. The observed lower zones of inhibition for the n-hexane extract suggest that the non-polar compounds present in *S. mombin* may have limited effectiveness against the tested isolates. This aligns with the work of Trusheva et al. [34] who have reported that the varying antimicrobial activities of different solvent extracts from *S. mombin*, indicating that the choice of solvent can significantly influence the extraction of bioactive compounds with antimicrobial properties. In contrast, the aqueous extract of *S. mombin* showed the highest effectiveness against tested isolates, with larger zone diameters of inhibition. This suggests the extract contains water-soluble bioactive compounds, such as alkaloids, flavonoids, phenols, and tannins, which have antibacterial properties [13].

The MIC and MBC values of S. mbin extracts against wound sample isolates was explored tonassay for antimicrobial potential and effective concentration for specific bacterial strain. The extracts showed a MIC value of 50 mg/ml against *P. aeruginosa*, with a MBC value of 100 mg/ml, suggesting a higher concentration is needed for bactericidal effects, aligning with previous studies on *S. mombin* extracts [35]. *S. mombin* extracts showed lower MIC and MBC values against *S. epidermidis* compared to *P. aeruginosa*, indicating lower antibiotic resistance. These results suggest lower concentrations are needed for effective inhibitory and bactericidal effects. The study found that *S. mombin* extracts have moderate antimicrobial activity against *S. aureus* strains, with a MIC value of 25 mg/ml for aqueous and methanol extracts, and 50mg/ml for n-hexane extract. Table 10 provides insights into the MIC and MBC values of *S. mombin* extracts against isolates derived from urine samples, including *E. coli*, *S. aureus*, *S. saprophyticus*, *T. vaginalis*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*. The MIC and MBC values ranged from 25 mg/ml to 100 mg/ml, indicating varying levels of susceptibility among the tested isolates. The range of MIC and MBC values

suggests variations in the effectiveness of the extracts against different pathogens. These findings are consistent with previous studies that have reported variable susceptibility patterns of bacterial isolates to *S. mombin* extracts [35, 12].

The qualitative analysis of *S. mombin* extracts revealed various phytochemical classes, including alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins, steroids, and tannins, which are known for their antimicrobial and antioxidant properties against antibiotic-resistant bacterial isolates. These compounds can contribute to the observed antimicrobial effects of *S. mombin* extracts against antibiotic-resistant bacterial isolates [13]. Anthraquinones were not detected in methanol and n-hexane extracts, suggesting they may not be the main antimicrobial constituents, possibly due to the extraction process and solvents. Saponins were also detected in the aqueous and methanol extracts but were not found in the n-hexane extract. Saponins are known for their diverse biological activities, including antimicrobial, anti-inflammatory, and anticancer properties [36]. The presence of saponins in *S. mombin* extracts further supports their potential as antimicrobial agents against multidrug-resistant bacterial isolates. The comprehensive analysis of bacterial isolates obtained from wound and urine samples, as well as the evaluation of *S. mombin* extracts, provided valuable insights into the prevalence of antibiotic-resistant bacteria and the potential antibacterial properties of the plant.

CONCLUSION

Antibiotic-resistant bacteria pose a global health challenge, necessitating novel therapeutic approaches. *S. mombin* plant extracts offer potential for developing new antimicrobial agents, highlighting the importance of exploring natural products and their bioactive compounds to combat antibiotic resistance and improve public health.

RECOMMENDATION

Further research is needed to identify bioactive compounds responsible for antimicrobial activity, understand their mechanisms, and conduct in vivo experiments and clinical trials for potential therapeutic applications.

LIST OF ABBREVIATIONS

CAZ- Ceftazidime
CRX - Cefuroxime
GEN - Gentamycin
CTR - Ceftriazone
ERY - Erythromycin
CX - Cloxacillin
OFL - Ofloxacin
AUG - Augmentin
CXM - Cefixime
NIT- Nitrofurantoin
CPR - Cefpirome
MSA - Mannitol salt agar
CLED - Cysteine lactose electrolyte-deficient.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ETHICAL APPROVAL

The ethical approval for this study was obtained from the Ethics and Research Section of Ondo State Ministry of Health (NHREC/18/08/2016).

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MKO and AOO managed the analyses of the study and were in charge of direction and planning.

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Molecular Detection of Extended Spectrum Beta-lactamases Among Clinical Isolates of *Pseudomonas aeruginosa* from Patients Attending Two Selected Hospitals in Niger State, Nigeria.

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ABSTRACT

Pseudomonas aeruginosa belongs to the ESKAPE group of superbugs and has been implicated in both healthcare-associated infections and as an opportunistic infection affecting patients with debilitating underlying conditions. The study aims to determine the prevalence of extended spectrum beta-lactamases (ESBL)- producing *P. aeruginosa*, antibiogram and associated risk factors for acquisition of *P. aeruginosa* infection whilst using conventional PCR to detect the molecular presence of ESBL genes (particularly *CTX-M*, *TEM* and *SHV*) among isolates of *P. aeruginosa* from patients attending Federal Medical Centre, Bida and General Hospital, Minna, Niger State, Nigeria. A total of 200 samples of wound swabs, pus, urine, ear and eye swabs were analyzed by standard bacteriological methods. Screening and confirmation of ESBL were done phenotypically by Kirby-Baur method of antibiotic susceptibility testing and Double Disk Synergy Testing (DDST), respectively. Molecular detection of ESBL genes: *CTX-M*, *TEM* and *SHV* was done using conventional PCR technique. Of a total of 200 samples collected, 27 (13.5%) were isolates of *P. aeruginosa*, 14 (51.9%) confirmed positive for ESBL using DDST and 12(44.4%) confirmed positive for the presence of one or more of the targeted ESBL gene using the conventional PCR technique. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (PPV) of DDST given as 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard against DDST technique. Prevalence of *CTX-M* (499kbp), TEM (862kbp) and SHV (827kbp) genes were distributed as 9(40.9%), 8(36.4%) and 5(22.7%), respectively. Prevalence of ESBL producing *P. aeruginosa* is high thus endemic in this region. A high prevalence in occurrence of *CTX-M* reinstates the changing pattern of ESBL producing *P. aeruginosa* in this geographical region. Therefore, this calls for a public health concern and highlights the need to develop an active surveillance system to track the spread and activating a robust Infection prevention and control measure.

Keywords: Niger State; Extended Spectrum; Beta-Lactamases; Molecular Detection; *Pseudomonas aeruginosa*

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INTRODUCTION

The World Health Organization (WHO) has classified *P. aeruginosa* as tier one priority pathogen of urgent concern, and new drugs are urgently needed as a result of the emergence of multi-drug resistance [1]. The frequency of *P. aeruginosa* infections, its clinical challenges and poor outcomes associated with these infections, and in particular the proportion of *P. aeruginosa* isolates that are resistant to antibiotics have resulted in these bacteria being classified as one of a group of six pathogens (the ESKAPE pathogens) that are the most problematic to treat [2]. As typical of ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.), drug-resistant nosocomial pathogens such as *Pseudomonas aeruginosa* poses unwavering and increasing global threats as they can be extensively drug resistant and pan drug-resistant [3]. *P. aeruginosa* that has become resistant to the carbapenem as well as the cephalosporins class of antibiotics as classified by the WHO is one of the three "Priority 1: Critical" groups of bacteria for which new treatment strategies are most critically needed [2]. Due to the recent COVID-19, there has been increased usage of antibiotics as well as hand sanitizers and disinfectants worldwide, thus accelerating the spread of cross resistance, this stems largely from the treatment of secondary underlying infections with the use of antibiotics [1,4]. Moreover, *P. aeruginosa* has been found to be the primary causative agent of nosocomial morbidity and mortality in people with Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD) and non-CF bronchiectasis [3] a report showed that by 2050 if no action has been taken, mortality and morbidity from Antimicrobial Resistance (AMR) will surpass any acute or chronic illnesses, including heart diseases and cancer with an estimated annual mortality of 10 million cases and cost estimates as high as US $100 trillion worldwide [3,5].

In Nigeria, *P. aeruginosa* is one of the most common pathogens implicated in all hospital acquired infections and one of the most common causes of post-operative wound infection (burns, wounds etc.) [6]. It has also been found to be one of the most common pathogens associated with both ventilator-associated pneumonia and catheter-associated urinary tract infections [2,4,6]. *P. aeruginosa* is an effective opportunistic pathogen unlikely to cross healthy, intact anatomical barrier, thus its infectiousness results from invasive medical procedures or weakened host defences. These predisposing conditions include debilitating illness, immuno-suppressant medication or intravenous injections. Once in the tissues, these bacteria express virulent factors including exotoxins and also causes endotoxic shock [7].

Extended spectrum β-lactamases (ESBL) hydrolyzes oxyimino-aminothiazole cephalosporins (cefotaxime, cefuroxime, cefepime, ceftriaxone and ceftazidime) as well as penicillins and other cephalosporins, excluding cephamycins [6]. Extended spectrum beta-lactamases were first reported in Germany, and persistent exposure of bacterial strains to myriads of beta-lactams has caused a dynamic and continuous production and mutation of lactamase (into a new beta lactamase called ESBLs) in many bacteria, thus expanding their activity even against the third and fourth-generation cephalosporins [6]. The most common and predominant ESBL in *P. aeruginosa* are the Ambler class A SHV-, TEM, CTX, VEB-, PER- and GES-type enzymes and the OXA-type enzymes from Ambler class D [8–10]. It has been reported that the presence of ESBLs confers a higher level of resistance to extended spectrum cephalosporins than AmpC, impermeability and efflux pump hyper-expression combined [3,11]. However, the production of ESBLs confers resistance at various levels to expanded spectrum cephalosporins [12–14]. These enzymes are encoded by different genes located on either chromosomes or plasmids as such ESBL-producing bacteria may not be detectable by the routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure [12–14]. Although studies abound in Nigeria on the prevalence of multi-drug-resistant *P. aeruginosa,* there is paucity of data on the molecular epidemiology of ESBL gene in Niger State. This study aims to detect the presence of Extended Spectrum Beta Lactamase (ESBL) among clinical isolates of *P. aeruginosa* of patients attending selected government hospitals in Niger State and also to determine the prevalence of ESBL genes *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ using conventional PCR among clinical isolates of *P. aeruginosa.*

METHODOLOGY

Study Design and Sample Size

This was a descriptive cross-sectional study conducted between July 2023 to December 2023 among patients attending Federal Medical Centre Bida, Niger State and General Hospital Minna, Niger State, North Central Nigeria. The Sample size (200) was calculated by Leslie Fisher's formular and the prevalence used was 13.5% from a similar study conducted in Minna, Nigeria. Participants were recruited by convenient or purposive sampling and inclusion criteria involving patients diagnosed with bacterial infection and those who consented to the research. Written informed consent was obtained from each

participant before questionnaire administration, while those unwilling were excluded. A semi-structured interviewer-administered questionnaire was used to obtain data on socio-demographics and associated factors predisposing individuals to ESBL infection. Ethical approval with ethical clearance number FMCB/HCS/HREC/APPR/VOL 2/39/23, HMB/GHM/136/VOL.III/653 was obtained from the Research Ethics Committee of both Federal Medical Centre Bida and General Hospital Minna, respectively.

Sample Collection

Approval was obtained in August and October for both General Hospital and FMC Bida respectively with sample collection beginning immediately. Specimens such as wound, pus, urine and ear swabs were collected from the participants and processed at the Medical Microbiology Laboratory of both hospitals following standard microbiology procedures.

Laboratory Methods and Analysis

Pseudomonas aeruginosa was identified as catalase positive, oxidase positive and produced greenish pigmentation on Nutrient agar and Cetrimide agar. The Kirby-Baur method was used in the antibiotic susceptibility test for *P. aeruginosa*. Double Disk Synergy Test (DDST) was done using CLSI guidelines for Co-amoxiclav (20/10 µg), Ceftazidime (30 µg) and ceftriaxone (30 µg) (Fidson & Co, Nigeria German Chemicals, Glaxo, Swipha, Abtex and Oxiod UK).

DNA extraction of *P. aeruginosa* was done by taking fresh colonies from the nutrient agar slant, suspended in 200 µl of TE buffer and turbidity was adjusted according to 0.5 McFarland's standard. The procedure for DNA extraction was performed according to RUN MEI DNA Extraction Kit Manual, China. PCR amplification was done using Transgen Biotech 2×EasyTaq PCR SuperMix, China. The bla_{SHV}, bla_{TEM} and bla_{CTX-M} primers (Table 1) and thermocycling protocols were used as previously described. The PCR conditions for all reactions involved an initial denaturation for 5minutes at 94°C followed by a final denaturation of 35 cycles for 30seconds (with specific annealing at 56°C for CTX-M and 53°C for TEM and SHV for 30seconds) followed by a final extension at 72°C for 35 cycles in 5 minutes. A total volume of 50 µl reaction mixture was used to amplify each of the bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes separately. The Polymerase Chain Reaction (PCR) amplified products were detected by 1% agarose gel electrophoresis at 80V for 30 minutes. Primers were optimized to assess Tm and TA. DDST evaluation was done in comparison with PCR as gold standard. Performance evaluation was done using markers such as true positives, true negatives, false positives and false negatives to determine sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV). The study was analyzed using SPSS version 25 software.

Table 1: Sequences of primers that were used [27].

Primer	Sequence (5'-3')	Product Size (bp)	Annealing (˚C)
***bla*SHV (F)**	ATTTGTCGCTTCTTACTCGCC	827	53
***bla*SHV(R)**	TTCACCACCATCATTACCGACC		
***bla*TEM (F)**	GTGCGCGGAACCCCTATT	862	53
***bla*TEM (R)**	GGGATTTTGGTCATGAGATTATC		
***bla*CTX-M (F)**	CGATGTGCAGTACCAGTAA	499	56
***bla*CTX-M (R)**	TAAGTGACCAGAATCAGCGG		

RESULTS

Table 2 shows the distribution for age, gender and place of residence. Also, 16 out of 27 (59.3%) isolates of *P. aeruginosa* are male, while 11 out of 27 (40.7%) are female with a *P-value* of 0.978.

Table 3 and Figures 2, 3 and 4 show the prevalence of ESBL among clinical isolates of *P. aeruginosa* was 14(51.9%) and 12(44.4%) for both DDST and PCR respectively. Table 3 shows Figure 1 show a prevalence of 13.5% representing 27 *P. aeruginosa* isolates out of a total of 200 samples.

the sensitivity, specificity, PPV and NPV of DDST given as 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard.

Table 2: Socio-demographic characteristics of clinical isolates *Pseudomonas aeruginosa* and respondents

Variables	*Pseudomonas aeruginosa* N=27 (n, %)	Participants N=200 (n, %)	*P-value*
Age Groups			0.998
Children (0-12)	3(11.1)	22(11.0)	
Adolescents (13-17)	1 (3.7)	7(3.5)	
Adults (>18)	23 (85.2)	171(85.5)	
Gender			0.978
Male	16 (59.3)	119(59.5)	
Female	11(40.7)	81(40.5)	
Place of residence			0.987
Urban	19 (70.4)	141(70.5)	
Rural	8(29.6)	59(29.5)	

Key: *=Statistically significant (i.e. < 0.05) n = number of isolates, N = Total number of respondents

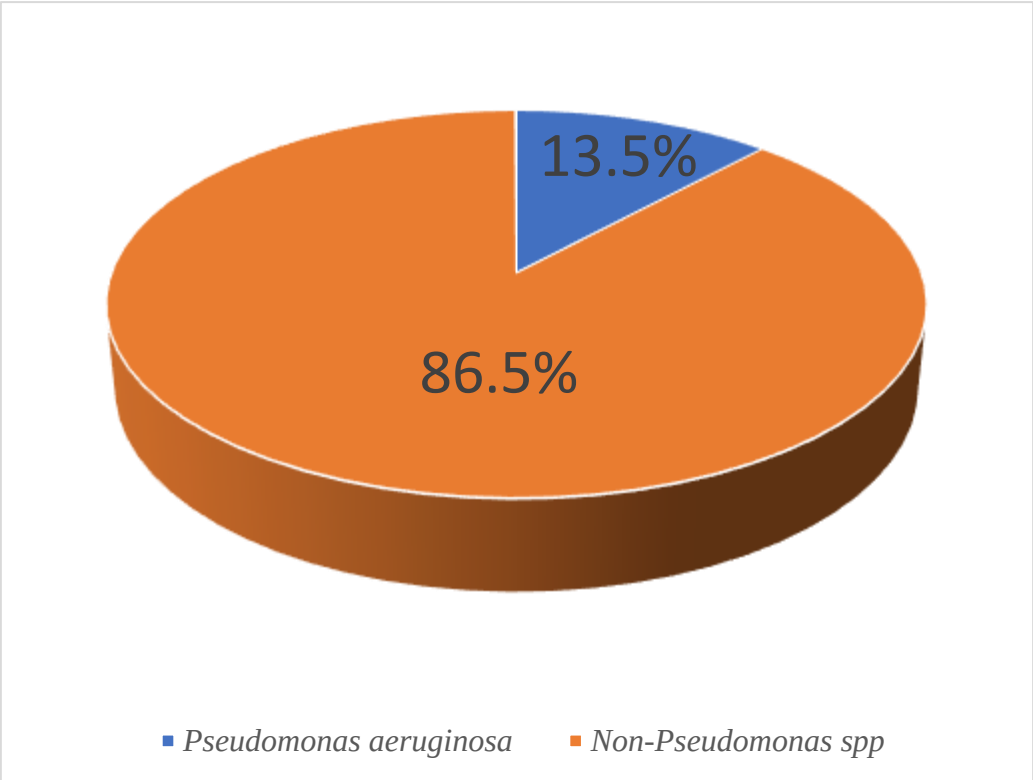

Figure 1: Percentage of confirmed *Pseudomonas aeruginosa* isolates

Table 3: Comparison of ESBL isolates using PCR and DDST

Variables	PCR		Total
	Positive (n, %)	Negative (n, %)	
DDST			
Positive	7(58)	7(47)	14
Negative	5(42)	8(53)	13
Total	12(100)	15(100)	27(100)

Key: n = number of isolates

Figure 2: Photograph of CTX-M PCR product (Positives: S19, S21 and 23). PC-Positive control; NC-Negative control;D-DNA ladder; Target gene: *blaCTX-M* (499kbkbp)

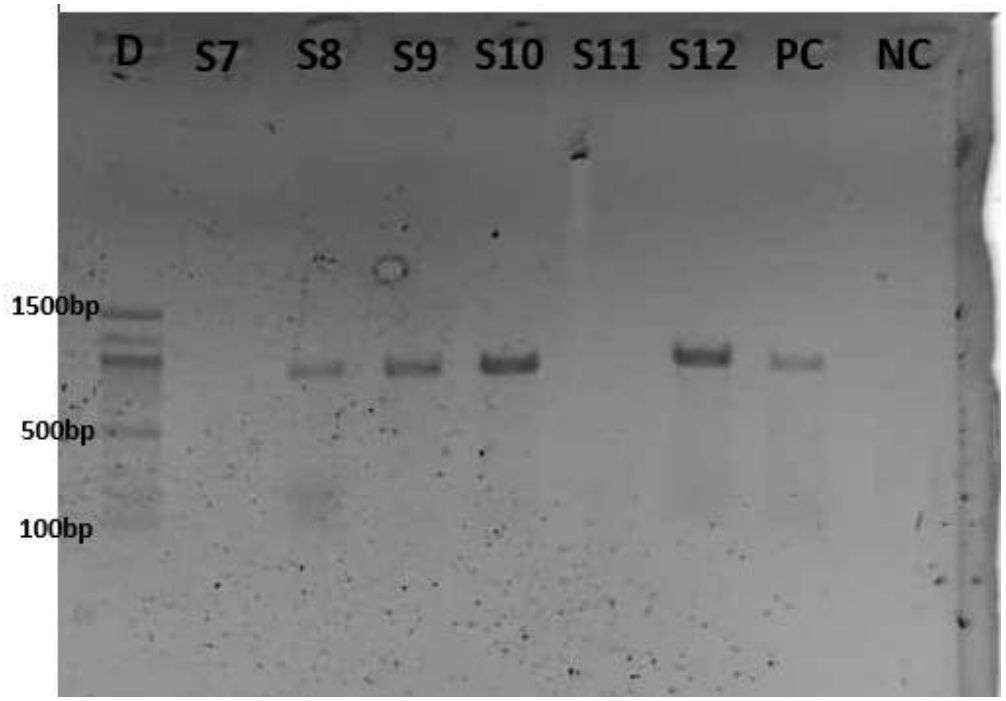

Figure 3: Photograph of TEM PCR product (Positives: S8, S9, S10, S12 and S13). PC-Positive control; NC-Negative control; D- DNA ladder; Target gene: *blaTEM* (862kbp)

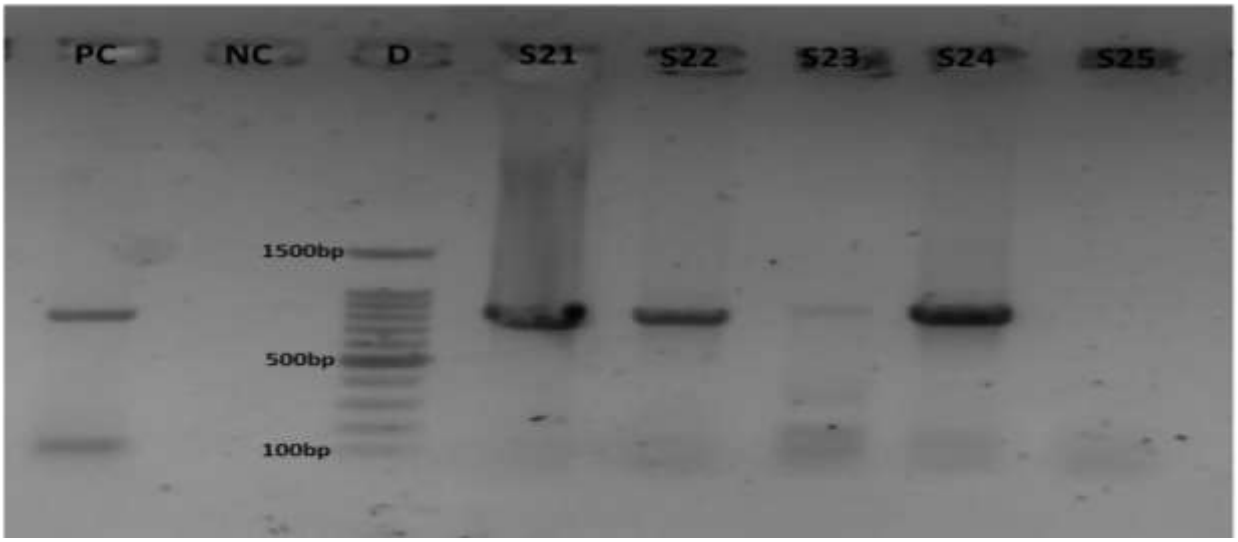

Figure 4: Photograph of SHV PCR product (Positives: S21, S22, S23, S24 and S25). PC-Positive control; NC-Negative control; D- DNA ladder; Target gene: *blaSHV* (827kbp).

Table 4 shows the antibiogram profile of *P. aeruginosa* isolates to various antibiotics and that Imipenem had the highest sensitivity. The highest sensitivity of 15(55.6%) was observed against Imipenem followed closely by Ciprofloxacin and Gentamycin with both having 8(29.6%) respectively. However, the highest resistance was observed against Cefotaxime and Cefoxitin with both sharing 21(77.8%) followed by Ceftriaxone, Tazobactam/Piperacillin and Ceftazidime with 18(66.7%), 14(51.9%) and 13(48.1%) respectively. Susceptibility pattern is shown in Table 4, with maximum resistance occurring against Cefoxitin (71.4%), Cefotaxime (64.3%), and Ceftriaxone (50%) of isolates. Table 5 and Figures 2, 3 and 4, depict the PCR result which showed the highest percentage in the co-occurrence of CTX-M+TEM followed by CTX-M+SHV with 5(18.5%) and 3(11.1%) respectively.

Table 4: Antibiogram profile of *Pseudomonas aeruginosa* isolates to various antibiotics

S/No.	Antibiotics	Sensitive (n, %)	Intermediate (n, %)	Resistant (n, %)
1.	Imipenem (10µg)	15(55.6)	4(14.8)	8(29.6)
2.	Ciprofloxacin (5µg)	8(29.6)	6(22.2)	13(48.1)
3.	Cefoxitin (30µg)	1(3.7)	5(18.5)	21(77.8)
4.	Ceftazidime (30µg)	8(29.6)	6(22.2)	13(48.1)
5.	Gentamycin (10µg)	4(14.8)	10(37.0)	13(48.1)
6.	Cefotaxime (30µg)	1(3.7)	5(18.5)	21(77.8)
7.	Ceftriaxone (30µg)	2(7.4)	7(25.9)	18(66.7)
8.	Tazobactam/Piperacillin (100/10µg)	4(14.8)	9(33.3)	14(51.9)
9.	Co-Amoxiclav (20/10µg)	8(29.6)	9(33.3)	10(37.0)

Key: n = number of isolates

Table 5: Distribution of CTX-M, TEM and SHV among *Pseudomonas aeruginosa* isolates

Distribution of ESBL among isolates

S/No	ESBL genes (n=27)	Frequency (n, %)
1	*bla*TEM	2(7.4)
2	*bla*SHV	1(3.7)
3	*bla*CTX-M + *bla*TEM	5(18.5)
4	*bla*CTX-M + *bla*SHV	3(11.1)
5	*bla*CTX-M + *bla*SHV + *bla*TEM	1(3.7)
	Total	**12(44.4)**

Distribution of Single ESBL genes

S/No	ESBL genes (n=27)	Frequency (n, %)
1	*bla*CTX-M	9(40.9)
2	*bla*TEM	8(36.4)
3	*bla*SHV	5(22.7)

Key: n = number of isolates

DISCUSSION

Although a significant portion of those found positive for *P. aeruginosa* were male, the relationship was not statistically significant. However, it was previously reported in Maiduguri and Minna that a significant relationship exists between *P. aeruginosa* acquisition and the male gender [6,7]. The high prevalence of *P. aeruginosa* shows an increasing endemicity in this part of the country buttressing the research carried out by previous authors [7,15,16].

ESBL producing *P. aeruginosa* were screened and confirmed using both DDST and conventional PCR technique respectively, as such, a prevalence of 51.9% for DDST and 44.4% for PCR was reported ESBL among clinical isolates of *P. aeruginosa* in this current study. While it is arguably true that variations in study designs may affect the result and outcome especially for genomic surveillance of ESBL and its types, the prevalence of the studied isolates are not in agreement with the study in Jos by Ejaz [17] who identified the prevalence of 7.2% for ESBL-producing *P. aeruginosa* in North-Central Nigeria. These findings are not in agreement with the work of Umar et al. [15] in Sokoto who identified *P. aeruginosa* in 36.7% of wound isolates and identified multi-drug resistance based on the antibiogram data. The study however did not go further to determine the phenotypic and molecular presence of ESBL. These findings are in agreement with the work of Olowo-Okere and colleagues [18] as well as Oli et al. [19] who reported a prevalence of 65% and 55.7% respectively. However, it is important to note a prevailing endemicity of ESBL in North-Central part of the country. Also, it is important to note that the present study is hospital-based research as such community-wide research might reveal an even higher and daunting prevalence. Both studies also used both DDST and PCR technique to obtain their findings. Similarly, the findings of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of DDST were 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard. This study is not in agreement with the reports of two studies in Ibadan [20,21] where the sensitivity, specificity, PPV and NPV were determined as 100%, 98%, 96% and 100% respectively. The difference could be attributed to the prevalence of co-occurrence of non-Class A ESBL genes not covered by this study. However, in the current study, the result indicates a low sensitivity for the detection of the 3 target genes thus buttressing the prevalence of false positives and false negatives associated with the phenotypic technique [22]. While it is true that the PCR technique is highly specific and sensitive, it targets only the genes probed, whereas ESBL is not only encoded by these 3 genes aforementioned earlier and DDST does not differentiate between the classes of ESBL. Due to the inexpensive nature of the DDST compared to PCR it is however, recommended for use in local settings or low-cost areas.

In the present study, a low resistance was recorded against Imipenem, Ciprofloxacin and Ceftazidime for all isolates of *P. aeruginosa.* Resistance was highest in Cefoxitin, Cefotaxime and Gentamycin with 71.4%, 64.3% and 42.9% respectively. Similarly, a high resistance was recorded for co-amoxiclav with 42.9% which further emphasizes the possibility of the *P. aeruginosa* having more than the three dominant genes detected. However, the high frequency of

resistance among the aforementioned cephalosporins may not be unconnected to the indiscriminate use and misuse of cephalosporin antibiotics and in addition to the fact that these antibiotics have existed much longer in circulation. These findings are in agreement with a study in Bangladesh [23] and another study in Anambra [19] as well as in Minna [7], with the identification of the various antibiotic profiles of ESBL producing *P. aeruginosa,* thus ensuring the right drugs is given for the right treatment ultimately preventing therapeutic failure. Moreover, the low resistance and high sensitivity observed against Imipenem might be due to the fact that this antibiotic is of the 4th generation cephalosporin and expensive, as such abuse and misuse are usually minimal. Although ESBL production was observed in slightly above 50% of the clinical isolates in this present study, resistance to 3rd generation cephalosporin was indicative of more non-class A ESBL gene carried by the clinical isolates. It is evident that the varied resistance mechanisms of this organisms has increased its adaptability to varying environmental conditions thus yielding varying antibiotic pattern [3,24].

The present study highlights a significant increase in the prevalence of CTX-M gene (40.9%), thus taking the lead with TEM (36.4%) and SHV (22.7%) following behind accordingly. This study is in agreement with another hospital based research on the prevalence of ESBL *P. aeruginosa* in paediatric patients focusing on only this three dominant genes which was carried out in Saudi Arabia [17]. However, this study does not agree with the findings of Rahimi et al. [25], where TEM (48.2%), SHV (28.2%) and CTX-M (18.8%) gene occurred frequently in that order among isolates of *P. aeruginosa* in Iran. Similarly, a study in south eastern part of Nigeria revealed a frequency of TEM(55%), CTX-M(45%) and SHV(35%) among clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* [26]. The differences in climatic conditions, geography and lifestyle pattern might have been associated with the differences.

The variation in the prevalence of ESBL-producing *P. aeruginosa* upon confirmation by PCR technique in this present study is evidence of the possible existence of non-class A ESBL and carbapenemases genes not sought by this study. The high occurrence of CTX-M can be explained by the changing patterns previously observed in Europe and Asia that may now have caught up with this part of Nigeria. The detection of multiple carriage of two or more beta-lactam genes within the same organism was reported in this study as well as numerous other studies [16,20,26] with CTX-M leading at 40.9% with SHV (36.4%) and TEM (22.7%) following closely. This study is in agreement with the work of Ejaz, in Saudi Arabia [17]. However, this present study does not agree with the findings of Rahimi and colleagues in Iran [25] as well as that of Ugbo and colleagues in Ebonyi [26]. The prevalence of CTX-M and co-occurrence amongst other genes such as in CTX-M+TEM followed by CTX-M+SHV with 5(18.5%) and 3(11.1%) respectively can be attributed to an existing relationship between antibiotic resistance and multiple carriage of resistant genes in addition to differences in climatic conditions, geography and lifestyle pattern earlier reported in other continents that might now have caught up with this part of Nigeria.

CONCLUSION

Our study highlights a high prevalence of ESBL among clinical isolates of *P. aeruginosa* thereby affecting the susceptibility pattern (antibiogram) of the isolates. Imipenem was found to be the most effective in its activity against *P. aeruginosa* isolates despite being the last drug of choice for treating multidrug resistance. The high cost of imipenem and the fact that AMR is gradually eroding the gains in the antibiotic production industry is worrisome. The relatively high prevalence of ESBL recorded following screening and confirmation by both phenotypic and molecular techniques showed the predominance of the target genes with CTX-M leading while TEM and SHV follows closely. Co-occurrences of CTX-M/TEM/SHV was recorded frequently and buttresses the relationship of antibiotic resistance with multiple resistant gene carriage. The assessment of DDST and PCR reveals the need for a robust diagnostic system for the detection of ESBL among *P. aeruginosa*. While PCR remains a gold standard for gene identification, it is relatively expensive and does not account for genes not included in the probe.

Therefore, it is pertinent to curb the spread of this organism by ensuring adequate adherence to standard operating procedures in the routine testing of ESBL in not just *P. aeruginosa* but other Gram-negative organisms. The economic burden of antimicrobial resistance (AMR) is gradually impoverishing Nigeria and the continent of Africa at large. As such the roles of infection prevention control measures and antibiotic stewardship will go a long way in surveillance, genomic mapping, by providing information for policies and improving treatment outcomes.

LIST OF ABREVIATIONS

µg/ml - microgram/millilitre
Amp C - Ampicillin Resistant Gene
Kbp – Kilobase Pairs
CLSI - Clinical and Laboratory Standard Institute
CTX-M - Cefotaxime Resistance Gene
DNA - Deoxyribonucleic Acid
Esbls - Extended Spectrum Beta Lactamases

GES - Guyana Extended Spectrum Beta Lactamases
IPAC – Infection Prevention And Control
OXA- Oxacillinase Gene
PCR - Polymerase Chain Reaction
PER - *Pseudomonas* Extended Resistance Gene
SHV - Sulphahydryl Variable
SPSS - Statistical Package for Social Science
TEM - Temoniera (Greek Patient) Gene
UK - United Kingdom
VEB - Vietnam Extended-Spectrum-Beta Lactamases
WHO - World Health Organization

AUTHOR CONTRIBUTIONS

HIC being the lead author participated in the research design, collation of data, manuscript preparation and overseeing most of the experiments including sample collection and statistical analysis. KM participated in the review of study design and overall manuscript review. MY contributed towards review of research design and provision of some materials used in the research experiments. MKG contributed largely in the laboratory workflow design and supported in the preparation of laboratory reagents used during the experiments.

CONFLICTS OF INTEREST

There authors have no conflict of interest to declare.

FUNDING

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ETHICAL APPROVAL

Ethical approval with ethical clearance number FMCB/HCS/HREC/APPR/VOL 2/39/23, HMB/GHM/136/VOL.III/653 was obtained from the Research Ethics Committee of both Federal Medical Centre Bida and General Hospital Minna, respectively. Informed consent was obtained from all participants and guardians of participants below the age of 16 years.

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