GET Journal of Biosecurity and One Health

GET JOURNAL OF BIOSECURITY AND ONE HEALTH

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Global Emerging Pathogens Treatment Consortium (GET)

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Message from the Editor-in-Chief

Prof. Akin Abayomi
Editor-in-Chief

Dear Esteemed Readers,

It is my distinct pleasure to welcome you to Volume 4, Issue 1 of the GET Journal of Biosecurity and One Health. Since its establishment in 2021, the journal has steadily grown into a platform for disseminating research that addresses pressing challenges at the intersection of biosecurity, environmental health, food systems, and the broader One Health agenda. Over the past few years, the journal has continued to attract contributions from scholars and practitioners committed to advancing knowledge that strengthens health security and promotes sustainable interactions between humans, animals, and the environment.

The current issue presents a diverse collection of studies that reflect the interdisciplinary nature of the One Health framework. The articles featured in this edition address important themes, including microbial ecology, vector-associated contamination, veterinary public health, food safety, and the application of geospatial methods in disease surveillance. Together, these contributions reinforce the importance of integrated scientific approaches in addressing complex public health challenges.

Among the studies featured in this issue is an investigation into the antibacterial activity of aqueous garlic (Allium sativum) extract against bacterial isolates recovered from the body surface of the American cockroach (Periplaneta americana) in Lagos, Nigeria, highlighting the potential role of natural plant-derived compounds in addressing microbial contamination associated with urban pests. Another article examines health professionals' knowledge of amniotic membrane banks in Côte d'Ivoire, offering valuable insights into awareness levels and capacity considerations surrounding emerging biomedical practices within healthcare systems.

This issue also includes a cross-sectional study on the prevalence and distribution of ectoparasites among domestic and stray cats in Sokoto State, Nigeria, providing important information for veterinary health management and zoonotic disease prevention. Complementing these studies is a geospatial investigation titled "Mapping the Prevalence of Chronic Kidney Disease using a Community-Centric Approach with Grid-Based Tessellation: An Example of CKDu Incidences in Northern Yobe State, Nigeria," which demonstrates how spatial analytics and community-centered mapping can contribute to improved understanding of disease patterns and support public health decision-making. In addition, the issue features research examining the effects of storage on the proximate composition, mineral content, and mycoflora of sundried pigeon pea (Cajanus cajan) seeds, providing insights relevant to food safety, agricultural storage practices, and nutritional quality.

Collectively, the studies presented in this issue underscore the critical importance of multidisciplinary collaboration in addressing emerging health threats and environmental challenges. By integrating perspectives from microbiology, veterinary science, environmental health, food science, and spatial epidemiology, this edition contributes to the growing body of knowledge necessary for advancing the goals of the One Health movement.

On behalf of the editorial board, I extend my sincere appreciation to our authors for their valuable contributions, our reviewers for their rigorous and constructive evaluations, and the editorial team for their dedication to maintaining the quality and integrity of the journal. Their collective commitment continues to strengthen the GET Journal of Biosecurity and One Health as a credible and accessible platform for scholarly communication.

As an open-access journal, we remain committed to ensuring that scientific knowledge is freely available to researchers, practitioners, and policymakers worldwide. We encourage continued submissions from scholars across disciplines whose work contributes to strengthening biosecurity systems, promoting environmental sustainability, and improving global health outcomes.

Thank you for your continued support of the GET Journal of Biosecurity and One Health. Together, through research, collaboration, and knowledge sharing, we can continue to advance solutions that safeguard the health of people, animals, and ecosystems.

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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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.

Dr. Adewunmi Akingbola is a medical doctor from Lagos State University College of Medicine, also an infectious diseases epidemiologist trained at the University of Cambridge. He has over 7 years of experience leveraging epidemiological expertise to tackle the menace of infectious diseases in Nigeria under the aegis of HealthDrive Nigeria, a project he founded where he conducts free tests, and highly-subsidized vaccinations, especially against Viral Hepatitis B. His groundbreaking research at Cambridge on comparing complete case analysis and multiple imputation techniques in estimating Hepatitis C prevalence emerged as the winning research of the Cambridge Public Health Early Career Researcher Competition in 2024. Dr Adewunmi Akingbola has served as a peer-reviewer for PLOS ONE, Frontiers of Public Health, Journal of Rare Diseases, and Discover Public Health journals. He was one of the three invited judges in the Oxbridge Acadian Scholars 3-minute research competition where he judged the works of 18 scholars from Oxford and Cambridge. He has presented his research works at several national and international conferences including the Cambridge West Hub for Pandemic Preparedness and One Health Event, Cambridge Annual Infectious Diseases Symposium, Cambridge Public Health Conference, and more recently, the United Kingdom Health Security Agency 2025 conference which engages over 1500 epidemiologists and public health professionals. He is a recipient of the 2020 AfriSAFE Community Hero awards by HSENations, the 2021 Princess Diana Awards, and more recently, he was named one of the 12 scientists globally to receive the prestigious 2024 Passion in Science Awards by New England BioLabs, Boston, USA, amongst others.

Kehinde Adebiyi is a Researcher in Microbiology at Indiana University, Bloomington, specializing in molecular microbiology, bioinformatics, public health, and sustainable development. He is a 2024 Stier Fellow and Konetska Fellow at IU's Department of Biology. Previously, he was a founding scholar at the Nigerian University of Technology and Management (NUTM), where he engaged in interdisciplinary research and innovation, including serving as a visiting scholar at 54gene. Kehinde's work in global health and policy advocacy has earned him recognition as one of the Top 100 BeatingCorona Heroes in Africa and a recipient of the Union Bank RISE Award for his role in combating COVID-19.

He founded one of Nigeria's first campus-based SDGs groups, expanding to over ten institutions, later leading the United Nations Academic Impact Millennium Fellowship, which impacted over 800,000 people across 20 countries. His Microbes for SDGs project bridges microbiology and sustainability, promoting microbial solutions to global challenges. He serves on the Board of Trustees for the Sustainable Development Goals Awareness Initiative, sits on the board of HealthDriveNG, and co-leads Policy Shapers, Africa's leading youth-led policymaking platform. Kehinde holds a First-Class Microbiology degree from Lagos State University and a Postgraduate Diploma from NUTM. He has published multiple papers, co-authored a book, and remains committed to advancing sustainability and science-driven policymaking.

Dr Ademolu Adegbenga is a medical doctor with over 25 years post-qualification clinical research experience in both the public and private sectors with over 15 years of the experience in a managerial position. He had published thirty-three peer-reviewed articles. Twenty-nine in the field of Endocrinology and Metabolism, one in healthcare management. Twenty-eight of his publications are in international journals, of which twenty are in American peer reviewed literature, one in European peer reviewed literature and six in Asian peer reviewed literature. He had published four books and written two book chapters. He holds a Masters of Science degree in Chemical pathology from the University of Lagos. His research and publications had earned him an international recognition and award by winning the 2023/2024 American based Marquis Publication Board "Who"s Who " in the world award. He is a well sort after international speaker and also editorial board member of American endocrine journals. He served as the Lead Guest Editor for the United States of America based International Journal of Diabetes and Endocrinology between 2019-2020 publishing a special issue on "hypoglycemia in diabetes" for the journal. He had been a reviewer for the Journal of Clinical Endocrinology and Metabolism (JCEM) of The Endocrine Society since 2014 and in the last four years he had been in the editorial board of the journal. During this period of serving, he had reviewed many National Institute of Health (NIH) funded research work with other reviewers for publication in JCEM, the official journal of the Endocrine Society, including a National Institute of Diabetes and Digestive and Kidney Diseases(NIDDK) sponsored research work done by a group of researchers in Massachusetts General Hospital, Boston, Massachusetts in which he was invaluable as a reviewer according to the authors comment among other NIDDK sponsored research work. He had served in the editorial board of many international journals. He is currently in the editorial board of Nigerian Journal of General Practice. Dr Ademolu was awarded the highest award of the Association of Nigerian Private Medical Practitioner as a "Distinguished Medical Practitioner" (DMP) in 2024.

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Activity of Garlic (*Allium sativum*) Bulb Against Bacterial Isolates from Body Wash of American Cockroach (*Periplaneta americana*) from Lagos, Nigeria

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ABSTRACT

Cockroaches have huge health importance; they may be involved in the passive mechanical transfer of pathogens from humans to animals and food. The study aimed to isolate the external bacterial flora of *Periplaneta americana* and to determine the antimicrobial activity of crude garlic extract and conventional antibiotics on the bacterial population. A total of 45 Cockroaches (n=45) were collected aseptically from Ojo and Iyana-era all in the Ojo local government area of Lagos State, Nigeria. Bacteria were isolated on nutrient agar, MacConkey agar, and Salmonella-Shigella Agar and were putatively identified based on cultural, morphological, and biochemical characteristics. The identification system was complemented with an Analytical Profile Index (API). A total of 30 bacterial strains were identified, of which 23 were Gram-positive, and 7 were Gram-negative, belonging to the *Pseudomonas, Bacillus, Streptococcus, Escherichia, Staphylococcus, Actinomycetes, Enterococcus, Serratia, and Listeria* genera. The antimicrobial activities of garlic extract on isolates revealed that strain OJ9, IE4, OJ8, and OJ3 had inhibition zone values of 26.5 ± 2.1, 26.0 ± 1.4, 24.5 ± 2.1, and 24. ± 1.4. A high inhibition zone was also obtained with strains OJ5, OJ10, and OJ4, respectively, whereas OJ1, OJ2, and OJ12 did not show inhibition zones. The antibiotic susceptibility and resistance patterns showed that Strain OJ1 was susceptible to ciprofloxacin but resistant to gentamycin and zinnacef, whereas OJ7 was susceptible to erythromycin and perfloxacin but resistant to other antibiotics, including. Also, strain IE7 was susceptible to perfloxacin, rocephin, and septrin. The study provided clear insight into the antimicrobial potency of the *Allium sativum* crude extract.

Keywords: Cockroach; Crude-garlic extract; Antimicrobial activity; Allicin; *Staphylococcus; Bacillus; Pseudomonas.*

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INTRODUCTION

Cockroaches are among the most widely distributed insects found in homes, food-handling establishments, hospitals, and health care facilities. They are also found in septic tanks where they feed on faecal matter. Aside from this, Cockroaches also feed on garbage and decaying foods [1]. They are vectors capable of transmitting pathogens to food, kitchen utensils, and different parts of homes. Various pathogenic microorganisms have been associated with cockroaches, which they convey on their cuticles or ingest, or excrete on surfaces. Some medically important bacteria have been isolated from the body surfaces of *Periplaneta americana*, some of which include *Staphylococcus* sp., *Streptococcus* sp., *Salmonella* sp., *Shigella* sp. *Escherichia* sp. *Campylobacter* sp, *Pseudomonas*, and *Klebisiella* sp. [2,3,4].

Among cockroach species, *Periplaneta americana* is consumed in oriental countries like China, where it is bred in large populations, sold to farmers and the general public as feed for livestock, or eaten as food [5]. *Periplaneta americana* is also reared by some farmers because they are found to be useful in the pharmaceutical industry. The insects have been used in drug formulations to cure several ailments such as gastroenteritis, duodenal ulcer, and pulmonary tuberculosis. The insect is also effective in relieving sore throat, fluid build-up, and tonsillitis due to its detoxifying properties [6].

Cockroaches are known for their vectorial capacity to distribute microbial pathogens carried on their body surfaces as mechanical carriers. Such a microorganism is dependent on the environment within which the cockroach circumnavigates. This makes it necessary to characterize the bacteria carried in each environment. Since cockroaches are also being promoted as possible sources of feed for poultry and other consumptive uses, it would be imperative to either eliminate the microbial fauna on the insect's body surface or render them ineffective in causing and spreading disease. Also, there is a need to protect the laboratory and other workers who work with cockroaches from potential infection from the microbial load on the insect's body surface. The acclaimed antimicrobial and insecticidal potency of some botanicals may be relied upon to ensure the safe handling and consumption of cockroaches, such as garlic (*Allium sativum*).

The antibacterial properties of garlic have been known for a very long time. Garlic has been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria, including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*, among others [7]. Farmers involved in breeding cockroaches also use garlic to disinfect the body surface, making the insects less harmful for consumption. These farmers use garlic to eliminate and prevent microbes and pathogens from contaminating the insects intended for processing as a source of food for livestock and humans [8]. However, the effectiveness of the treatment has not been established, just as little is known about the species spectrum of the cockroach biota used. This calls for the present study, aimed at identifying the bacterial flora on the body surface of *Periplaneta americana* collected from Lagos and investigating the antibacterial activities of Garlic (*Allium sativum*) crude aqueous extract on bacterial isolates from *P. americana*.

METHODOLOGY

Sampling Sites and Sample Collection

In order to determine the bacterial community composition of the body wash of *Periplaneta americana* (Cockroach), septic tanks of selected residential buildings of Iyana-Era and Ojo, Ojo Local Government Area of Lagos State were sampled. In total, 45 live Cockroaches were aseptically collected by handpicking between 1 and 3 a.m. from the two sampling sites. Thirty cockroaches were collected from Ojo, and 15 from Iyana-Era septic tank using a direct collection approach and stored in a sterile large bottle. Samples were transported to the laboratory in the morning for microbial enumeration.

Microbial Enumeration

The bacterial community composition of *Periplanata americana* was estimated and isolated using the standard plate count technique. Serial dilution of 10-fold of samples using physiological saline was carried out, and a 0.1 ml aliquot was transferred onto nutrient agar, McConkey agar, and Salmonella-Shigella agar. The spread plate technique was used. Petri plates were incubated at 35 ± 2 °C for 18-24 hrs. Colonies were purified by sub-culturing on nutrient agar.

Identification and Characterization of Bacterial Isolates

Isolates were characterized and identified based on their colonial and cellular morphology, biochemical characteristics, following the taxonomic scheme of Cowan and Steel [9] to identify isolates to the genus level. The biochemical test was complemented with an analytical profile index (API V4.0 for Staph, 50 CH and 20 E) to identify the predominant members to the species level. Some of the substrates tested on the isolates were O-nitrophenyl-β-D-galactopyranoside, Arginine, Lysine, Ornithine, Sodium thiosulphate, Urea, Tryptophan, Creatine sodium pyruvate, Mannitol, Innositol, Sorbitol, Maltose, and Arabinose, etc.

Antimicrobials

Activity of Crude Garlic Extract on Selected Isolates

One hundred grams of garlic bulb were peeled, washed **a**nd transferred into a washed electrical blender after which 125 ml of distilled water was

added and blended into a paste. The paste was passed through a wire mesh to sieve out the shaft. The filtrate was passed through Whatman No 1 filter paper. The extract was further purified by passing it through a membrane filter (0.45 µm, Millipore). The crude garlic extract was stored in a sterile, dark bottle and refrigerated. A standard solution of the bacterial concentration with the equivalent of 0.5 Mcferland (1.5 x10^8 cfu/ml) was prepared of which 1ml aliquot was poured on sterile solidified Mueller-Hinton agar plate, decanted and air-dried. A sterile cock borer was used to punch out plugs (discs) on the seeded agar plate adjacent to one another. A 50 µL filter-sterilized crude garlic extract was transferred into the wells and incubated at 35 ± 2ºC for 18-24 hrs. The diameter of the clear zones was measured in millimeters. Agar plates were prepared in duplicates.

Antibiotic Susceptibility Patterns of the Isolates
Antibiotic susceptibility and resistance patterns of the isolates were determined by disc agar diffusion methods following guidelines established by Bauer *et al.* [10]. Isolates were tested against Gram-positive and Gram-negative multidisc. The antibiotics tested with their respective concentrations were Pefloxacin (10 µg), gentamycin (10 µ g), ampicillin (30 µg), Zinnacef (20 µg), amoxacillin (30 µg), rocephin **(**25 µg**),** ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg), and erythromycin (10 µg). The antibiotic disc was placed on Mueller-Hinton agar plates already seeded with 0.1 ml inoculum of the bacterial isolates, and the petri-plates were incubated at 35 ± 2 ºC for 18-24 hrs and observed for zones of inhibition. The antibiotic resistance and susceptibility patterns were interpreted according to the Clinical and Laboratory Standards Institute Guidelines [11].

RESULTS

The Cultural Morphological and Biochemical Identification of isolates is shown in Table 1. Also, Table 2 depicts the Analytical Profile Index (API) of predominant isolates obtained from the body of a cockroach. Culture-dependent analysis of the bacterial flora of the body of *Periplaneta americana* identified 30 bacterial isolates. Of the 30 bacterial isolates identified, 23 were Gram-positive and 7 were Gram-negative. A total of 10 genera of isolates were identified with various numbers of strains, viz., *Pseudomonas* sp. (8), *Streptobacillus* (1), *Actinomyces* (4), *Bacillus* (3), *Streptococcus* (2), *Enterococcus* (1), *Staphilococcus* (6), *E. coli* (1), *Serratia* (3), and *Listeria* (1). Bacterial strains from Ojo were designated as OJ1, OJ2, OJ3, OJ4, OJ5, OJ6, OJ7, OJ8, OJ9, OJ10, OJ11, OJ12 and OJ13 whereas bacterial strains from Iyana Era were designated as IE1, IE2, IE3, IE4, IE5, IE6, IE7, IE8, IE9, IE10, IE11, IE12, IE13, IE14, IE15, IE16 and IE17. Strain OJ1 is a Gram-negative rod with irregular edges, flat smooth surfaces, catalase and oxidase positive, and motile. Strain OJ3 has a circular, creamy colour with flat, smooth surfaces and a Gram-positive, motile rod, which tested positive for catalase and oxidase but does not produce indole nor utilize lactose. Bacterial strain OJ10 is a Gram-positive coccus with circular, raised, smooth surfaces, catalase and oxidase positive, which ferments lactose.

Bacterial strain IE1 is a pinkish circular, smooth, motile rod cell bacterium, catalase and oxidase positive, that does not produce hydrogen sulphide gas. Also, the bacteria strain IE13 is a creamy Gram-positive coccus, catalase- and oxidase-positive. The API analysis revealed that strain OJ3 fermented D-glucose, D-fructose, and D-mannose but did not utilize maltose, xylitol, D-mellibiose, raffinose, or xylose. Bacterial strains OJ5, OJ11, and IE12 were able to utilize plant-derived glycogen, such as methyl-D-mannoside, methyl-G-glucoside, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, and cellebois. while OJ9 and OJ11 tested negative for alcohol sugars such as xylitol and sorbitol.

The abundance of various bacterial isolates from *Periplaneta americana* is depicted in Figure 1**.** *Pseudomonas* sp. is the most abundant in the Ojo sample, with a prevalence of 38%, compared with the 12% recorded for the Iyana Era sample. Also, *Staphylococcus* was high in the Iyana Era sample, at 30%, compared with less than 10% in the Ojo sample. *Escherichia*, *Enterococcus*, and *Streptobacillus* genera were isolated from Ojo, whereas *Serratia* and *Listeria* were identified from Iyana Era samples.

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Table 1: Cultural, Morphological, and Biochemical Identification of Isolates

Isolates codes	**Colonial morphology**				**Gram staining**	**Cellular morphology**	**Catalase**	**Oxidase**	**Indole**	**Motility**	**Citrate**	**Lactose**	**Gas**	**H_2S**	**Glucose**	**Putative identity**
	Colour	Shape	Elevation	Edge												
OJ1	Green	Irregular	Flat	Smooth	-	Rod	+	+	-	+	+	-	-	-	+	*Pseudomonas* sp.
OJ2	Green	Irregular	Convex	Smooth	-	Rod	+	+	-	+	+	-	+	+	+	*Pseudomonas* sp.
OJ3	Cream	Circular	Flat	Smooth	+	Rod	+	+	-	+	+	-	+	+	+	*Streptobacillus* sp.
OJ4	Cream	Circular	Raised	Rough	+	Rod	+	+	-	-	+	-	-	-	_	*Actinomyces* sp.
OJ5	Yellow	Irregular	Convex	Rough	+	Rod	+	+	-	-	+	-	-	+	+	*Bacillus* sp.
OJ6	Green	Circular	Flat	Smooth	-	Rod	+	+	-	+	+	-	-	+	+	*Pseudomonas* sp.
OJ7	Green	Irregular	Flat	Smooth	-	Rod	+	+	+	+	+	-	+	+	-	*Pseudomonas* sp.
OJ8	Yellow	Circular	Raised	Smooth	+	Rod	+	+	+	+	+	-	+	+	+	*Bacillus* sp.
OJ9	Whitish gray	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+	-	+	+	+	*Streptococcus* sp.
OJ10	Cream	Circular	Raised	Smooth	+	Cocci	+	+	-	+	+	+	-	+	+	*Enterococcus* sp.
OJ11	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+	-	-	+	+	*Staphylococcus* sp.
OJ12	Green	Irregular	Flat	Smooth	+	Rod	+	+	+	+	+	-	+	+	+	*Pseudomonas* sp.
OJ13	Cream	Circular	Flat	Rough	-	Rod	+	+	-	+	+	+	-	+	+	*E. coli*
IE1	Pink	Circular	convex	Smooth	+	Rod	+	+	-	+	+		+	-		*Serratia* sp.
IE2	Pink	Circular	Convex	Smooth	+	Rod	+	+	+	+	+		-	-		*Serratia* sp.
IE3	Green	Irregular	Convex	Smooth	-	Rod	+	+	+	+	+		-	-		*Pseudomonas* sp.
IE4	Green	Irregular	Convex	Smooth	-	Rod	+	+	+	+	+		-	-		*Pseudomonas* sp.
IE5	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+		+	-		*Staphylococcus* sp.
IE6	Pink	Circular	Convex	Smooth	+	Rod	+	+	-	+	+		+	-		*Serratia* sp.
IE7	Cream	Circular	Raised	Serrated	+	Rod	+	+	-	-	+		+	+		*Listeria* sp.
IE8	Cream	Circular	Convex	Smooth	+	Rod	+	+	+	-	+		+	-		*Bacillus* sp.
1E9	Whitish	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+		+	-		*Streptococcus* sp.
IE10	Yellow	Circular	Convex	Smooth	+	Bacilli	+	+	-	+	+		-	+		*Actinomyces* sp.
IE11	Cream	Circular	Raised	Rough	+	Cocci	+	+	+	+	+		-	-		*Staphylococcus* sp.
IE12	Yellow	Circular	Convex	Smooth	+	Bacilli	+	+	-	+	+		-	-		*Actinomyces* sp.
IE13	Cream	Circular	Raised	Smooth	+	Cocci	+	+	-	+	+		-	-		*Streptococcus* sp.

IE14	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+		+	-		*Staphylococcus* sp.
IE15	Whitish	Circular	Convex	smooth	+	Bacilli	+	+	+	+	+		-	+		*Actinomyces* sp.
IE16	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+		+	-		*Staphylococcus* sp.
IE17	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+		+	+		*Staphylococcus* sp.

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Table 2: Analytical profile index of the predominant isolate obtained from the body of the cockroach

Sugar fermentation	**OJ3**	**OJ5**	**OJ9**	**OJ11**	**IE12**
D-glucose	+	+	+	-	-
D-fructose	+	+	+	+	+
D-mannose	+	++	+	++	++
Maltose	-	+	-		++
Lactose	+	+	+		++
D-trehalose	+	+	+	++	++
D-mannitol	+		+	+	
Xylitol	-		-		
D-mellibiose	-	+	-		++
Potassium nitrite	+		+		
Naphthyl acid phosphate	+		+		
Sodium pyruvate	+		+		
Raffinose	-	+	-		++
Xylose	-		-		
Sucrose	+		-		
Methyl-D-glucoside	-		-		+
N-acethyl-glucosamine	+		+		++
Arginine	+	+	+	+	
Urea	+	-	-	-	
Glycerol	-	++		++	++
Erythritol					
D-arabinose		++		++	++
L-arabinose		++		++	++
Ribose		++		++	++
D-xylose		++		++	++
Adonithol					
Methylxyloside				+	
Galactose		++		++	++
Mannitol		+		+	
Sorbitol		+		-	
Methyl-D-Mannoside		+		+	
Methy-g-glucoside		+		+	
N-acetyl glucosamine		+		+	
Amygdalin		++		++	++
Arbutin		++		++	++
Esculin		++		++	++
Salicin		++		++	++
Celleboise		++		++	++
O-nitrophenyl-*β-D* galactopyranoside		+		+	+
Lysin		-		-	-
Ornithine		-		-	-
Sodium citrate		-		-	-
Sodium thiosulphite		-		-	-
Tryptophan		-		-	-
Creatine-sodium pyruvate		+		+	-
Kohn's charcoal gelatin		+		-	+
OX		-		+	+
Nitrite reduction		-		-	-
Motility medium		+		+	+
McConkey medium		+		+	+
OF-o		+		+	+

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The antimicrobial activity of garlic extract on isolates is shown in Table 3. High zones of inhibition were recorded with strain OJ9, IE4, OJ8, and OJ3 with values 26.5 ± 2.1, 26.0 ± 1.4, 24.5 ± 2.1, and 24. ± 1.4. High inhibition zones were also obtained with strains OJ5, OJ10, and OJ4, while OJ1, OJ2, and OJ12 did not show inhibition zones. A high zone of clearing indicates the potency and efficacy of aqueous garlic extract against microorganisms inhabiting the body surfaces of cockroaches.

Furthermore, the antibiotic susceptibility and resistance patterns of the isolates are depicted in Table 4. Strain OJ1 was susceptible to ciprofloxacin but resistant to gentamycin and zinnacef, whereas OJ7 was susceptible to erythromycin and perfloxacin but resistant to other antibiotics. Also, strain IE7 was susceptible to perfloxacin, rocephin, and septrin.

DISCUSSION

Cockroaches are found in human habitation, such as environments where food is stored, processed, or served [1]. Aside from these environments, they are found in hospitals, including intensive care units, theatres, and laboratories [12, 13]. Also, they visit septic tanks, drains, and bathrooms. Additionally, they are nocturnal and omnivorous. These attributes make them ideal carriers of pathogens and parasites, including bacteria, protozoans, fungi, viruses, and helminths [14,15]. In this study, 30 bacterial species belonging to 10 genera, namely *Pseudomonas, Bacillus, Streptococcus, Escherichia, Staphylococcus, Actinomycetes, Enterococcus, Streptobacillus, Serratia, and Listeria*, were studied. were identified. Some of these bacterial genera have been reported from cockroaches [16]; however, this is the first report of their isolation from septic tanks in Ojo and Iyan-Era.

Bacillus has been reported as the most predominant in various studies, as shown in Adeleke *et al*. [16] and Isaac *et al*. [17]. *Pseudomonas*, however, was the most abundant in this study. *Staphylococcus* was also dominant in the Iyana-era sample. This genus has been implicated in the causation of certain nosocomial infections. Though various bacteria, viruses, and fungi are known to cause nosocomial infections, *Staphylococcus aureus* is known to be among the most common. In this way, it is complemented with *Escherichia coli*, and other microbes not isolated in this study [18,19,17]. It is not surprising that *E. coli* was isolated from the Iyana-Era sample, as it is a hardy organism that can survive harsh environments. Most members of the organisms isolated in the study belong to the family Enterobacteriaceae, which are normal inhabitants of the human intestine. Their abundance in the cockroach body may have resulted from cockroaches interacting with human fecal matter.

Table 3: Antimicrobial activities of crude garlic extract against bacterial isolates

S/N	Isolate codes	Zone of inhibition
		(Mean ± SD)
1.	OJ1	0.00
2.	OJ2	0.00
3.	OJ3	24.0 ± 1.4
4.	OJ4	16.0 ± 1.4
5.	OJ5	22.5 ± 2.1
6.	OJ6	4.0 ± 5.7
7	OJ7	0.00
8.	OJ8	24.5 ± 2.1
9.	OJ9	26.5 ± 2.1
10.	OJ1O	19.5 ± 0.71
11.	OJ11	17.0 ± 2.8
12.	OJ12	0.00
13.	OJ13	12.5 ± 2.1
14.	IE1	16.0 ± 0.0
15.	IE2	14.0 ± 1.4
16.	IE4	26.0 ± 1.4
17.	IE6	8.5 ± 1.2
18.	IE7	17.5 ± 2.1
19.	IE11	6.5 ± 4.2
20.	IE12	15.0 ± 1.4
21.	IE16	15.0 ± 0

Spices are used globally to enhance flavors and preserve perishable foods [20]. Spices are simply any dried, fragrant or aromatic vegetable or plant substances that enhance flavors. Different spices used on a daily basis have been documented to possess antimicrobial and medicinal values [21]. It is important to note that garlic belongs to one of the useful aromatic spices. The antimicrobial activities of garlic against Gram-positive and Gram-negative bacterial pathogens have been reported [22, 23, 24, 25, 20].

The results of the antimicrobial activities of garlic, based on the zones of inhibition recorded, are shown in Table 3. A high zone of inhibition was observed with bacterial strains IE1, IE4, OJ3, OJ4, OJ5, OJ8, OJ9, OJ10, and OJ11. The high inhibition zones observed on Mueller-Hinton agar plates seeded with the respective organisms indicate the efficacy and antimicrobial potential of the aqueous garlic extract. Our findings corroborate the earlier report of Safitri *et al*. [26], which shows the efficacy of aqueous garlic extract against *S. typhimurium*, S. *agalactiae*, S. *aureus*, and E. *coli.*

The study also explored the antibiotic resistance and susceptibility patterns of the isolates. Although antibiotics are not always used against cockroaches, highly resistant strains have been reported, with pathogens associated with food [27, 28]. Also, the great association between cockroach and food could be a probable reason for the isolation of resistant strains in food [1]. Bacterial strains were susceptible to pefloxacin, ciprofloxacin, rocephin, and erythromycin. It is important to note that isolates showing clear zones of inhibition to conventional antibiotics were also susceptible to aqueous garlic extract. The results of this study make it apparent that garlic or aqueous garlic extract can serve as an alternative antimicrobial to conventional antibiotics, thereby curbing the menace of mechanical transfer of pathogens by cockroaches in homes and other locations.

CONCLUSION

The study involves culture-independent analysis of the bacterial flora on the body surface of *Periplanata americana*. Ten bacterial genera were isolated, including *Pseudomonas*, *Staphylococcus*, and *Serratia*, which were among the dominant genera. The antimicrobial potential of the aqueous garlic extract against the isolates showed that the extract was highly effective against most bacterial isolates, with clear zones of inhibition observed, even compared with conventional antibiotics. Garlic extract has been shown to be highly effective at curtailing the mechanical transfer of pathogens that cockroaches can convey.

AUTHOR CONTRIBUTION

Denloye AA: Initiator and Principal Investigator (PI)
Alafia AO: Initiated Draft Zero of Manuscript
Ashade AO: Preparation of agar, plating, and identification of isolates
Ajelara KO: Technical Support on extract preparation and storage.
Godonu KG; Adetunji BH, Oke S, Babaniyi PI; Oke R; Ezun SJ; and Ajose RA: Took up the collection, maintenance, and preparation of body wash of experimental cockroach.
Oyefolu AOB: Identification of isolates and initial review of the Draft zero of the manuscript.

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Table 4: Antibiotic susceptibility and resistant patterns of the bacterial isolates.

No	Isolate code	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
1.	OJ1	S (22)	R (0)	R (0)	R (0)	R (0)	R (0)	S (20)	R (0)	R (0)	S (20)
2.	OJ2	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
3.	OJ3	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
4.	OJ4	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	I (17)	R (0)	R (0)	R (0)
5.	OJ5	R (0)	R (0)	R (0)	R (0)	R (0)	I (17)	S (26)	R (0)	I (17)	S (20)
6.	OJ6	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
7.	OJ7	S (23)	R (0)	R (0)	R (0)	R (0)	R (0)	I (15)	R (0)	R (0)	S (20)
8.	OJ8	S (25)	I (16)	R (0)	R (0)	R (0)	I (15)	S (25)	S (21)	I (15)	S (25)
9.	OJ9	S (21)	R (0)	R (0)	R (0)	R (0)	R (0)	S (20)	R (0)	R (0)	S (19)
10.	OJ10	S (20)	R (0)	R (0)	R (0)	R (0)	I (17)	S (28)	R (0)	R (0)	S (19)
11.	OJ11	S (24)	R (0)	R (0)	R (0)	R (0)	R (0)	S (24)	R (0)	R (0)	S (20)
12.	OJ12	R (0)	I (15)	R (0)	R (0)	R (0)	R (15)	R (0)	R (0)	R (0)	R (0)
13.	OJ13	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
14.	IE3	I (14)	R (0)	R (0)	R (0)	I (10)	R (0)	R (0)	R (0)	R (0)	I (15)
15.	IE4	I (17)	R (0)	R (0)	R (0)	I (13)	S (25)	I (19)	I (13)	S (24)	S (20)
16.	IE6	I (16)	R (0)	R (0)	R (0)	I (14)	R (0)	I (16)	S (20)	I (11)	R (0)
17.	IE7	S (23)	R (0)	R (0)	R (0)	I (12)	S (26)	S (20)	I (17)	S (20)	I (15)
18.	IE8	R (0)	R (0)	R (0)	R (0)	I (13)	S (21)	R (0)	I (11)	R (0)	R (0)
19.	IE9	I (15)	R (0)	R (0)	R (0)	I (13)	R (0)	I (19)	S (23)	I (12)	R (0)
20.	IE10	S (23)	R (0)	R (0)	I (15)	I (12)	S (27)	S (21)	I (17)	S (20)	I (16)
21.	IE15	S (25)	R (0)	R (0)	R (0)	I (10)	S (25)	S (20)	I (15)	S (23)	I (14)
22.	IE17	S (21)	R (0)	R (0)	R (0)	I (13)	I (18)	I (18)	I (19)	S (20)	I (15)

Key: Susceptible: 21mm and above, Intermediate: 11 -20 mm, Resistance: 0-10 mm

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Health Professionals' Knowledge of Amniotic Membrane Banks in Côte d'Ivoire

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ABSTRACT

The amniotic membrane is a thin membrane located on the inside of the placenta. It is widely used to treat several conditions. Its therapeutic value in the biomedical field requires the creation of a biological database. The creation of this bank requires the participation of healthcare staff. This study aimed to assess the knowledge of healthcare professionals about the biobank and the conservation of amniotic membrane for use in research and therapy in Côte d'Ivoire. A cross-sectional survey was conducted among healthcare professionals. The survey included questions on participants' professional profiles, their knowledge of the biobank, its role and activities, and the use of amniotic membrane tissue in research and therapy. A total of 215 people took part in the survey, 64.2% of whom said they had never heard of a biobank before. Only 35.3% of participants were aware of the role and activities of a biobank. Regarding the storage of amniotic membrane in a biobank for diagnosis, treatment, and research, only 32.1% of respondents were aware of this. Bivariate analysis showed that function (intern: OR=1.11; 95% CI [0.26- 4.35], midwife: OR=16.2; 95% CI [6.71-45.8], trainee: OR=19.4; 95% CI [4.55 109], and length of professional experience (1 5: OR=2.01; 95% CI [1.09- 3.82], "< 1 year": OR=12.4; 95% CI [2.41- 227],) were associated with knowledge of amniotic membrane preservation and its use for research and therapy. The healthcare professionals surveyed had limited knowledge of biobanks in general and amniotic membrane preservation in particular. However, the majority had a positive attitude towards amniotic membrane donation for research and therapeutic purposes.

Keywords: Knowledge; Biobank; Amniotic Membrane; Health Professional; Ivory Coas.

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INTRODUCTION

The development of the foetus is ensured by a complex system consisting of the umbilical cord, the amniotic fluid, and the placenta. The foetal membranes are made up of two layers: an outer layer (chorion) and an inner layer called the amniotic membrane (AM). The AM is a thin membrane on the inner surface of the placenta; it surrounds the foetus and delimits the amniotic cavity, which is filled with amniotic fluid [1,2]. The many years of research into the functions, structure, and properties of AM have led to several applications in the field of regenerative medicine. At the beginning of the 20th century, AM was first used by Davis for skin transplantation [3]. Subsequently, it was widely used in the treatment of burns [4,5], for surgical dressings [6,7], for surgical reconstruction of the bladder [8], and in the treatment of several other pathologies [9-13]. In view of the therapeutic interest of AM in the biomedical field worldwide, placental membrane banks have been set up in both developed [14] and developing countries [15].

Côte d'Ivoire, a developing country, has a Biological Resource Centre (CeReB) and a cell biology laboratory hosted by the Institut Pasteur. Despite its efforts in biobanking, the country has no AM bank. Setting up such a bank requires the participation of healthcare staff and people willing to donate their placenta and associated data. This is only possible if there is a good understanding of the issues surrounding the AM bank. To date, very few studies have assessed healthcare professionals' knowledge of these issues. The main objective of this study was to assess healthcare professionals' knowledge of biobanking and amniotic membrane preservation and its use in research and therapy.

METHODOLOGY

Setting and Type of Study

This was a cross-sectional study conducted from 28 July to 05 November 2022 at the Centre Hospitalier Universitaire de Cocody (CHU). The Centre Hospitalier Universitaire de Cocody is a third-level public referral hospital inaugurated in June 1970, located in Abidjan, Côte d'Ivoire. It became a Public Industrial and Commercial Establishment (EPIC) on 6 June 1984. The mission of the CHU de Cocody is to provide curative and preventive health care, initial and continuing training for health care staff, and medical, pharmaceutical, and odontological research. The CHU de Cocody has several medical departments, including the Gynaeco obstetrics and Paediatrics Unit. This department strengthens the healthcare offer by increasing the capacity to care for women, newborns, children and adolescents. It has 228 beds, including 112 inpatient beds, 23 emergency beds, and 18 intensive care beds.

Our study population was Healthcare professionals in the gynaecology-obstetrics department of the CHU of Cocody. All staff in the gynaecology-obstetrics department of CHU de Cocody were eligible to take part in the study. All healthcare professionals (hospital interns, gynaecologists, midwives, and trainees) belonging to the gynaecology-obstetrics department of the CHU de Cocody who were present at the time of the visit and who had given their consent were included in the study.

Form and Data Collection

Sampling was opportunistic. All eligible people were contacted during normal working hours over the study period. The questionnaire was administered to staff during a brief interview on the study's purpose.

Statistical Analysis

The data were entered and analysed using EpiData 3.1 and Stata 11, respectively. Descriptive analysis (univariate, bivariate) was used. Statistical tests (Pearson's Chi-square or Fisher) were used, at the 95% confidence level, to test the existence of a statistical link between each of the professional profile variables and the following variables: "knowledge of the role of a biobank" and "knowledge of the conservation and use of the amniotic membrane". The results are presented as Odds Ratios (ORs) showing the gross effects of 'knowledge of the role of a biobank' and 'knowledge of the conservation and use of the amniotic membrane' on the professional profile variables.

Ethical Conditions

Measures were taken to ensure the study was conducted ethically. In fact, the survey only began after obtaining authorization from the director of the CHU de Cocody. In addition, the information was collected with participants' consent and in strict confidentiality, and the results were reported anonymously and coded.

RESULTS

Occupational Characteristics of Participants The occupational characteristics of participants are shown in Table 1. A total of 215 people took part in the study, of whom 79.1% were women. The majority (63.30%) were midwives. The average age of professional experience was 5.37 ± 4.13 years, with a range of 0 to 20 years. More than half (50.6%) of the participants had more than 5 years' professional experience.

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Table 1: Respondents' Occupational Profile

Characteristics	Numbers	Percentage (%)
Functions of participants		
Hospital interns	25	11.6
Gynaecologists	41	19.1
Midwives	136	63.3
Interns	13	6
Sex		
Male	45	20.9
Female	170	79.1
Duration of work experience		
<1 year	17	7.9
1- 5 years	72	33.5
>5 years	126	58.6

General Knowledge of the Biobank

Just over a third (35.8%) of participants said they had already heard of a biobank, and just over half (54.7%) had heard of it for the first time during the training courses. As regards the role and activities of a biobank, 35.3% of participants provided an exact answer: the collection, processing, preservation, and provision of samples with their associated data. However, 67.4% were unaware of the importance of sample traceability in the biobanking process.

As for the Institut Pasteur de Côte d'Ivoire biobank, only 2.3% reported being aware of its existence. As for the regional biobank of ECOWAS countries, only 1.4% were aware of its existence. The bivariate analysis (Table 3) showed that function (internist: OR=1.60; 95% CI [0.49- 5.20], midwife: OR=18.3; 95% CI [7.89-47.2], reference = "gynaecologist") and length of professional experience ([1 5[: OR=2.01; 95% CI [1.09- 3.82], '< 1 year': OR=12.4; 95% CI [2.41-227]; reference = '>= 5years') were statistically associated with knowledge of the role of a biobank.

Knowledge of Amniotic Membrane Storage and Its Use in Research and Therapy

The results (Table 4) showed that none of the participants stored amniotic membranes within their healthcare establishment. As regards knowledge of amniotic membrane storage for research, diagnosis, and treatment, 67.9% said they were unaware of it. However, the majority (84.7%) would agree to donate the placental membrane for therapy and research purposes. Of the participants who refused to donate their amniotic membrane, just under half (48.50%) explained their refusal by cultural reasons.

Bivariate analysis (Table 5) showed that function (intern: aOR=1.11; 95% CI [0.26- 4.35], midwife: aOR=16.2; 95% CI [6.71-45.8], trainee: aOR=19.4; 95% CI [4.55-109], reference = "Gynaecologist") and length of professional experience ([1 5[: aOR=2.01; 95% CI [1.09- 3.82], '< 1 year': aOR=12.4; 95% CI [2.41- 227], reference = '>= 5years') were associated with knowledge of amniotic membrane preservation and its use for research and therapy.

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Table 2: General Knowledge of Biobanking

Variables	Numbers	Percentage (%)
Have you ever heard of a biobank?		
Yes	77	35.8
No	138	64.2
If so, where have you heard of it before?		
Mass media	12	15.6
Get to work	4	5.2
During a training course	42	54.5
From a health worker	3	3.9
With a loved one	2	2.6
More than one source of information	14	18.2
Knowledge of the role and activities of a biobank		
Yes	76	35.3
No	139	64.7
In your opinion, sample traceability Does it play a role in the biobanking process?		
Yes	70	32.6
No	145	67.4
Did you know that Côte d'Ivoire has a Biobank?		
Yes	5	2.3
No	210	97.7
Did you know that the Institut Pasteur de Côte d'Ivoire houses the regional biobank for ECOWAS member countries?		
Yes	3	1.4
No	212	98.6

Table 3: Knowledge of Biobank Activities by Occupational Profile

Characteristics	Knowledge of the role and activities of a biobank			Bivariate analysis		
	Yes n (%)	No n (%)	Total n (%)	OR[2]	95% CI[2]	p-value
Function						<0.001
Gynaecologists	33 (80%)	8 (20%)	41 (100%)	—	—	
Hospital interns	18 (72%)	7 (28%)	25 (100%)	1.60	0.49- 5.20	
Midwife	25 (18%)	111 (82%)	136 (100%)	18.3	7.89-47.2	
Trainee	0 (0%)	13 (100%)	13 (100%)	7.351	0.00, NA	
Duration of the experience professional						<0.001
>= 5 years	55 (44%)	71 (56%)	126 (100%)	—	—	
[1 5[	20 (28%)	52 (72%)	72 (100%)	2.01	1.09- 3.82	
< 1 year	1 (5.9%)	16 (94%)	17 (100%)	12.4	2.41- 2.27	

1 n (%)
2 OR = Odds Ratio, CI = Confidence Interval
NA = not applicable

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Knowledge of amniotic membrane storage and its use in research and therapy

The results (Table 4) showed that none of the participants stored amniotic membranes within their healthcare establishment.

As regards knowledge of amniotic membrane storage for research, diagnosis, and treatment, 67.9% said they were unaware of it. However, the majority (84.7%) would agree to donate the placental membrane for therapy and research purposes. Of the participants who refused to donate their amniotic membrane, just under half (48.50%) explained their refusal by cultural reasons.

Bivariate analysis (Table 5) showed that function (intern: aOR=1.11; 95% CI [0.26- 4.35], midwife: aOR=16.2; 95% CI [6.71-45.8], trainee: aOR=19.4; 95% CI [4.55-109], reference = "Gynaecologist") and length of professional experience ([1 5[: aOR=2.01; 95% CI [1.09- 3.82], '< 1 year': aOR=12.4; 95% CI [2.41- 227], reference = '>= 5years') were associated with knowledge of amniotic membrane preservation and its use for research and therapy.

Table 4: Knowledge of amniotic membrane conservation and its use for research and therapy, and the perception of donating amniotic membrane samples.

Variables	Numbers	Percentage (%)
Do you store placental membrane tissue?		
Yes	0	0
No	215	100
Did you know that the placental membrane is used for research purposes?		
Yes	95	44.2
No	120	55.8
Did you know that the amniotic membrane is held in a bank for diagnosis, treatment, and research?		
Yes	69	32.1
No	146	67.9
Would you be willing to donate placental membrane samples for scientific research?		
Yes	182	84.7
No	33	15.3
If not, why don't you want to do Donation of placental membrane samples?		
Cultural Reason	16	48.5
Personal Reason	8	24.2
Religious Reason	6	18.2
Cultural and Religious Reason	3	9.1

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Table 5: Knowledge of amniotic membrane conservation and its use in research and therapy, according to professional profile

Characteristics of the participants	Knowledge of the preservation of the amniotic membrane and its use for research and therapy purposes			Bivariate Analysis		
	Yes, n (%)	No, n (%)	Total, n (%)	OR[2]	95% CI[2]	p-value
Function						<0.001
Gynaecologists	35 (85%)	6 (15%)	41 (100%)	—	—	
Hospital interns	21 (84%)	4 (16%)	25 (100%)	1.11	0.26- 4.35	
Midwife	36 (26%)	100 (74%)	136 (100%)	16.2	6.71-45.8	
Trainee	3 (23%)	10 (77%)	13 (100%)	19.4	4.55-109	
Duration of the experience professional						0,020
>= 5 years	65 (52%)	61 (48%)	126 (100%)	—	—	
[1 5[	26 (36%)	46 (64%)	72 (100%)	1.89	1.05- 3.45	
< 1 year	4 (24%)	13 (76%)	17 (100%)	3.46	1.15- 12.8	

[1] n (%)

[2] OR = Odds Ratio, CI = Confidence Interval

DISCUSSION

This cross-sectional study provides an overview of the knowledge of healthcare professionals working in obstetrics and gynaecology regarding biobanks and the use of AM in research and therapy. Only 35.5% of participants were aware of the role and activities of a biobank. Regarding the use of AM in research and therapy, only 32.1% of respondents were aware of it.

The results of this study showed that the majority of participants (64.2%) had never heard of a biobank. These results corroborate those of Kintossou [16] and Chen [17], whose studies were conducted in Côte d'Ivoire and China, respectively. The limited knowledge of the biobank in the professional environment could be explained by the fact that it is a concept that is rarely discussed during medical training courses. In fact, only 19.5% claim to have heard of biobanking in Côte d'Ivoire. In the study by Kintossou [16], some participants defined the biobank as a savings structure reserved for biologists.

Although the participants in this study knew very little about the use of AM in the health sector, the majority stated they would be willing to donate a placenta. This suggests that healthcare professionals are willing to participate in establishing an AM bank in Côte d'Ivoire. Understanding potential participants' perceptions of biological sample donation is a fundamental step for the success of any AM sample and data collection campaign. The perception among healthcare professionals who refused to donate AM indicates the presence of religious and cultural barriers. Like blood donation, AM donation is certainly associated symbolically with cultural affiliations within which it is not given to a stranger [18].

Moreover, in many cultures, the future of AM is linked to the future of the human being who has just been born [19]. The creation of an AM bank will require the participation of motivated individuals, including healthcare professionals, who are prepared to donate biological samples and offer data [20,21]. Understanding potential participants' perceptions of AM donations will be a fundamental step toward successfully creating an AM bank.

This study had a number of limitations. The people included in our sample are not representative of all healthcare professionals in Côte d'Ivoire. Consequently, these results may not be extrapolable to the entire target population. Another limitation is that we did not ask participants whether they would agree to take part in setting up an AM bank. Finally, our statistical analysis led us to calculate only crude ORs, which could be subject to confounding bias.

However, these results are of particular interest insofar as we aimed to gather information on healthcare professionals' knowledge of the conservation and use of AM for research and therapeutic purposes. Qualitative studies are needed to understand the perceptions of healthcare professionals and the general public about AM donation.

CONCLUSION

Healthcare professionals' knowledge of amniotic membrane banks and their uses in the biomedical field is limited. There are also cultural and religious barriers to amniotic membrane donation among these professionals. Setting up such a bank will require taking into account both the country's socio-cultural particularities and the legal framework in force.

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AUTHORS' CONTRIBUTIONS
OKA designed, performed the methodology, and data analysis. The manuscript was written and edited by OKA, KKA, and NDM. OKA, KKA, and KKM collected the data. NDM participated in the data analysis. AA and DM participated in the study design and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

DECLARATION OF COMPETING INTEREST
The authors declare that they have no known competing financial interests or personal relationships that might appear to influence the work reported in this article.

LIST OF ABBREVIATIONS
CHU: Centre Hospitalier et Universitaire; AM: Amniotic membrane; CeReB: Biological Resource Centre; OR: Odds Ratio; Public Industrial and Commercial Establishment (EPIC); ECOWAS: Economic Community of West African States.

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Prevalence and Distribution of Ectoparasites in Domestic and Stray Cats in Sokoto State, Nigeria: A Cross-Sectional Study

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ABSTRACT
Ectoparasites are a significant cat health concern, particularly in regions with varying environmental and management conditions. This study assessed the prevalence and distribution of ectoparasites in domestic and stray cats in Sokoto State, Nigeria, with a focus on *Rhipicephalus sanguineus*, the brown dog tick. A cross-sectional study was conducted examining 30 cats, comprising 15 domestic and 15 strays. The presence of ectoparasites was determined through visual inspection and identification. The study identified *Rhipicephalus sanguineus* as the sole ectoparasite, with an overall prevalence of 13.3%. All infestations occurred in stray cats (26.7%), while no ectoparasites were found in domestic cats. The prevalence was significantly higher in females (25.0%) than in males (0.0%), and in adults (21.05%) than in juveniles (0.0%). The findings highlight a notable disparity in ectoparasite prevalence between domestic and stray cats in Sokoto State. The higher prevalence among stray cats, particularly females and adults, underscores the need for targeted control measures and public education to effectively manage ectoparasite infestations.

Keywords: Ectoparasites; *Rhipicephalus sanguineus*; Prevalence; Cats; Sokoto State; Nigeria.

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INTRODUCTION

Ectoparasites pose a serious threat to both domestic and stray animals, significantly affecting their welfare and resulting in several health problems. Animals with skin and fur infestations from parasites, including lice, fleas, and mites, may experience allergic reactions, skin irritation, and itching [1,2]. Animals that have ectoparasites may experience extreme discomfort and anguish, which may result in overgrooming and hair loss [3]. Ectoparasites not only have an immediate negative impact on the health of the host animal, but they can also spread serious zoonotic diseases to humans, including ehrlichiosis, rickettsiosis, tularemia, and Lyme disease [4, 5]. Several ectoparasites, such as lice (*Felicola subrostratus*), mites (*Demodex cati*, *Notoedres cati*), and fleas (*Ctenocephalides felis*), can infect cats in particular [6]. By inducing stress and compromising the immune system, ectoparasites can also have unintended consequences for an animal's health, increasing susceptibility to other illnesses [7]. Because they are present in the environment, ectoparasites also indirectly affect human health [8]. Increased rates of vector-borne illness transmission have been linked to high ectoparasite populations in communities, especially in locations with inadequate sanitation and hygiene standards [9]. Ectoparasite abundance, for example, is frequently associated with socioeconomic factors, such as overcrowding and substandard housing conditions, which favour their spread [10].

Public health problems may be made worse by this scenario, especially for disadvantaged groups with little access to healthcare. A tropical wet-and-dry climate characterises Sokoto State, situated in the far northwest of Nigeria. This has important implications for the occurrence of ectoparasites [11]. The state is bordered to the north by the Niger Republic, to the west by Kebbi State, and to the east by Zamfara State. Its total area is estimated to be 27,825 km². There are two seasons in the climate: a dry season from November to April and a wet season from April to October, with a peak in August. The region experiences 300–800 mm of rainfall on average each year, with April recording the highest temperatures of 40.6°C and December the lowest of 12.8°C [12]. Sokoto's climate, characterized by high temperatures, low humidity, and unpredictable rainfall, makes it difficult for animals to survive [12]. The lifespan and survival rates of ectoparasites can be influenced by these circumstances, which may result in increased infestations during the wet season when humidity levels rise. Furthermore, the region's primarily rainfed agricultural methods may affect the availability of hosts for ectoparasites, thereby further influencing the incidence of these parasites [13]. The majority of ectoparasite research conducted in similar regions has focused on the effects of these organisms on agricultural productivity and livestock health. Research has shown that ectoparasites, such as fleas and ticks, are common across a range of livestock species and play a substantial role in the spread of diseases that can negatively impact animal health and production [30]. The majority of prior studies on ectoparasites in dogs, small ruminants, poultry, and cattle have left a large knowledge vacuum on the incidence of ectoparasites in cats [31]. The objective of this research is to determine the different types and distribution of ectoparasites in domestic and stray cats in Sokoto State, Nigeria.

METHODOLOGY

Study Area

Sokoto State, which is in Nigeria's northwest, is bounded to the north by the Niger Republic, to the east by Katsina State, to the south-east by Niger State, to the south by Kwara State, and to the west by the Benin Republic. It occupies an area of roughly 62,000 km² and is located between latitudes 10°N and 13°58'N and longitudes 4°8'E and 6°54'E [14]. The Inter-Tropical Convergence Zone (ITCZ) regulates the interaction between the warm tropical marine air mass from the Atlantic Ocean and the dry, dusty tropical continental air mass from the Sahara Desert, thereby affecting the climate [14].

Collection and Identification of Ectoparasites

A total of thirty cats, fifteen domestic and fifteen stray, were randomly sampled for ectoparasites. The Usmanu Danfodiyo University Veterinary Teaching Hospital provided domestic cats, while stray cats were sampled in Sokoto city. Cats were enticed with fried fish and confined for analysis. Cats must be at least 2 months old to meet the inclusion criterion. The identification of domestic cats was based on customer interviews, whereas strays were classified based on the lack of ownership indicators such as collars or surgical scars [15,16]. The age and sex of every cat were noted.

Collection and Processing of Ectoparasites

Employing a fine-toothed flea comb and a thorough body search, ectoparasites were collected [15]. Similarly, after fleas and lice were discovered in the comb, ticks were manually extracted with blunt forceps and collected. Out of the thirty cats that were inspected throughout the harmattan season, only four ectoparasite specimens were found. Ticks were kept in specimen vials with 5% glycerol and 70% alcohol. The ectoparasites were examined and identified using stereomicroscopy at the Parasitology and Entomology Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

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Identification of Ectoparasites
Tick specimens were identified based on their morphological characteristics using identification keys and guides provided by [16] and [17]. The identification process involved comparing specimen morphology to known characteristics documented in these guides.

Data Analysis
The prevalence of ectoparasite infestation was calculated using the formula provided by [18]. The Chi-square tests were used to analyse the differences in prevalence across various age groups, sexes, and between domestic and stray cats [19]. Statistical analysis was conducted using STATA statistical software (StataCorp. Stata Statistical Software: Release 14. College Station, TX: StataCorp LLC).

RESULTS
A total of 30 cats were examined for ectoparasites. The only ectoparasite identified was the brown dog tick, *Rhipicephalus sanguineus*, with an overall prevalence of 13.3%. The prevalence was significantly higher in stray cats (26.7%) compared to domestic cats, where no cases were found (Tables 1 and 2). Additionally, the prevalence was notably higher in adult cats (21.1%) compared to juveniles (0.0%) (Table 4.3). Female cats also showed a higher prevalence (25.0%) than males (0.0%) (Table 4.4).

Table 1: Overall Prevalence of Ectoparasites in Cats in Sokoto

Cat type	Juvenile	Adults	Total
Domestic	11 (73.3%)	4 (26.7%)	15 (100%)
Stray	3 (20.0%)	12 (80.0%)	15 (100%)
Total	14 (46.7%)	16 (53.3%)	30 (100%)

Table 2: Prevalence of Ectoparasites in Cats by Type, Age, and Sex

Variable	Level	Negative	positive	Total	χ^2 (DF)	P-values
Cat type	Domestic	15 (100%)	0 (0.0%)	15 (100.0%)	4.6154 (1)	0.032
	Stray	11(73.3%)	4 (26.7%)	15 (100.0%)		
	Total	26 (86.7%)	4 (13.3%)	30 (100.0%)		
Age	Adult	15 (78.9%)	4 (21.1%)	19 (100.0%)	2.6721 (1)	0.102
	Juvenile	11 (100.0%)	0 (0.0%)	11 (100.0%)		
	Total	26 (86.7%)	4 (13.3%)	30 (100.0%)		
Sex	Female	12 (75.0%)	4 (25.0%)	16 (100.0%)	4.0385 (1)	0.044
	Male	14 (100.0%)	0 (0.0%)	14 (100.0%)		
	Total	26 (86.7%)	4 (13.3%)	30 (100.0%		

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Ectoparasites were found exclusively in stray cats (26.7%), with no infestation detected in domestic cats; the difference was statistically significant (p=0.032), indicating a higher risk among stray cats. Although adult cats had a higher prevalence of ectoparasites (21.1%) than juveniles (0.0%), the difference was not statistically significant (p=0.102). Additionally, female cats had a significantly higher prevalence (25.0%) than male cats, which were entirely free of infestation (p=0.044), suggesting a possible sex-related difference that warrants further investigation.

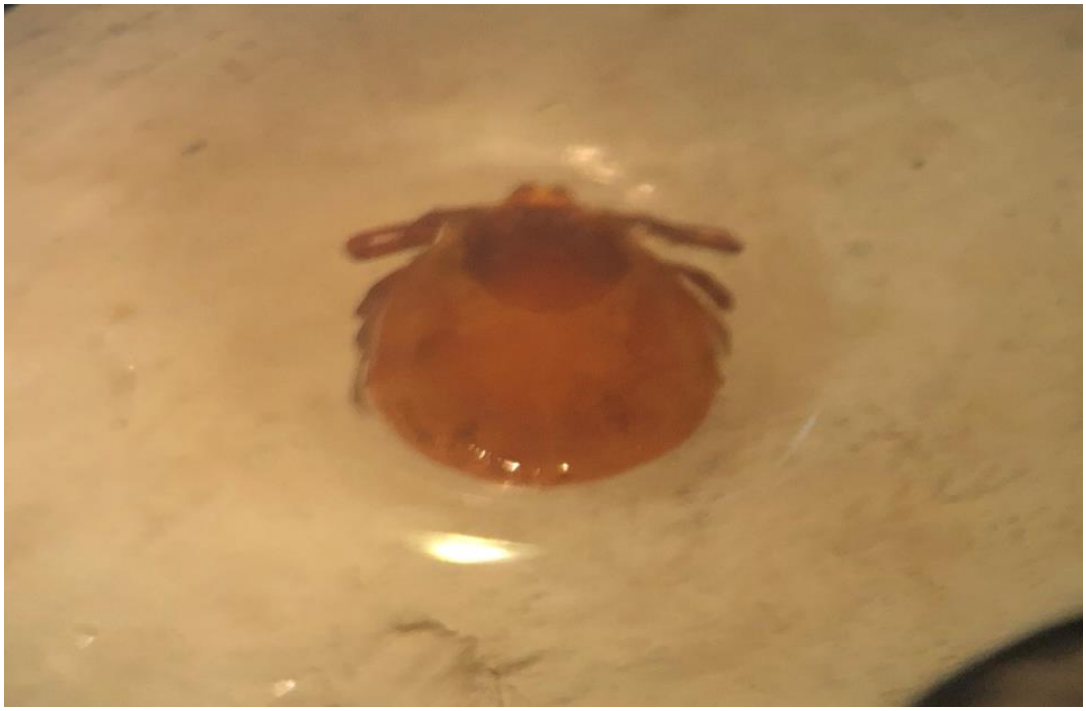

Figure 1: *Rhipicephalus sanguineus* as seen under a stereomicroscope (x20 magnification).

DISCUSSION

This study investigated the prevalence and distribution of ectoparasites in domestic and stray cats in Sokoto State, Nigeria, with a focus on *Rhipicephalus sanguineus*, the brown dog tick. The overall prevalence of ectoparasites (ticks) was 13.33%, with all infestations occurring exclusively in stray cats. None of the domestic cats examined harboured ectoparasites, indicating a significant difference between the two cat populations. The absence of ectoparasite(s) in domestic cats contrasts with findings from [32], who found that (95.5%) cats in Ekiti state, Nigeria, were infected with two or more ectoparasites. In addition, it contrasts with studies in other regions, where domestic cats have been reported to have higher prevalence rates, such as 37.1% in the United States [6] and 23.7% in the northeastern United States [23]. This difference could be attributed to better care and regular ectoparasiticide use among domestic cats in Sokoto State, or it might reflect a lower environmental burden of ectoparasites in the areas where these cats are kept.

The study found that stray cats had a significantly higher prevalence of ectoparasites (ticks) (26.67%) compared to domestic cats. This aligns with previous studies that found stray or free-roaming cats are more susceptible to ectoparasite infestations due to increased exposure to outdoor environments and a lack of regular veterinary care [20, 6]. The significantly higher prevalence in stray cats underscores the need for targeted ectoparasite control measures within this population. Interestingly, all ectoparasite-positive cats were female, while none of the male cats were infested. This finding is consistent with some previous studies [24, 25] that suggest sex may play a role in susceptibility to ectoparasite infestations. The reasons behind this gender disparity could include hormonal differences, behavioural factors, or differences in grooming habits between male and female cats. However, it is worth noting that other studies have found no significant difference in ectoparasite prevalence between males and females [26, 27].

Age also appeared to be an important factor, with adult cats showing a higher prevalence of ectoparasites (21.05%) compared to juveniles (0.00%). This result is consistent with findings by [28] and [24], who identified age as a critical risk

factor for ectoparasite infestation, likely due to the increased exposure and broader range of environments frequented by adult cats over time.

The limited presence of only *Rhipicephalus sanguineus* in this study contrasts with other research that has identified a wider variety of ectoparasites in cats, including fleas and lice [29, 21]. This could be due to geographical differences, the specific environmental conditions of Sokoto, or the limited sample size in this study.

The findings of this study showed the importance of addressing ectoparasite infestations in stray cats, particularly through community-based interventions and public education. The absence of ectoparasites in domestic cats suggests that regular veterinary care and the use of ectoparasiticides are effective preventive measures. However, the study's small sample size and focus on a single ectoparasite species may limit the generalizability of the results.

CONCLUSION

This study reveals a significant difference in ectoparasite prevalence between stray and domestic cats in Sokoto State, with stray cats being more heavily infested. The higher prevalence among female and adult cats highlights specific risk factors that could inform future control measures. Further research with a larger sample size and a broader focus on different ectoparasite species is recommended to develop a more comprehensive study of ectoparasite distribution in the region.

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DECLARATION OF COMPETING INTERESTS

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

AUTHOR CONTRIBUTIONS

Abubakar Yahaya Hassan: Conceptualization, Data Collection, Methodology, Resources, Validation, Writing, Original Draft, Writing, Review & Editing.
Mohammed Dalhatu Lawal: Conceptualization, Methodology, Resources, Supervision, Validation, Writing, Review & Editing.
Usman Mahmud: Supervision, Validation, Writing, Review & Editing.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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Mapping the Spatial Prevalence of Disease Using a Community-Centric Approach with Hexagonal Grid Tessellation: A Case of CKDu Incidences in Northern Yobe State, Nigeria

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ABSTRACT

Chronic Kidney Disease of unknown etiology (CKDu) poses a major public health challenge in Northern Yobe State, Nigeria, particularly in settings characterized by limited geocoded health records and weak disease surveillance systems. In such data-poor contexts, alternative methodological approaches are required to understand disease distribution and guide etiological investigations. This study demonstrates how CKDu spatial prevalence can be examined using a community-centric, GIS-based approach that relies on surveys of diagnosed CKD cases within victim households in a CKDu-endemic region of Yobe, integrating participatory data collection with open-source geospatial tools. Household-level data were collected through electronically administered questionnaires deployed via the KoboToolbox platform, supported by community leaders and implemented through snowball sampling over a 28-day period. Spatial analyses, including hexagonal grid-based tessellation, hotspot mapping, kernel density analysis, and spatial autocorrelation, were employed to visualize and quantify disease clustering. CKD incidence counts were aggregated within a uniform hexagonal grid, and spatial variability was classified using standard deviation–based incidence density categories to identify statistically meaningful concentration patterns across the study area. The analysis identified three distinct hotspots categorized by disease incidence density levels: high (7–10 cases), medium (3–6 cases), and low (1–2 cases). Spatial statistics derived from Moran's I index yielded a value of 0.1046, with a *z*-score of 4.95 and a *p*-value of 0.000001, indicating a less than 1% probability that the observed clustering of CKD incidences occurred randomly. Overall, the findings demonstrate that CKDu occurrence in Northern Yobe State is spatially non-random and can be effectively characterized through GIS-based, community-centric approaches in data-constrained regions. Thus, by generating spatially explicit evidence from diagnosed household cases, this methodology provides a robust foundation for targeted exploration of environmental risk factors and supports informed public health surveillance, environmental management, and policy interventions aimed at uncovering the aetiology of heightened CKDu prevalence in Northern Nigeria.

Keywords: Tessellation; Disease mapping; CKDu; Spatial prevalence; Qfield survey; Bade CKD

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INTRODUCTION

Chronic Kidney Disease of unknown Etiology (CKDu) has emerged as a significant global public health concern, predominantly affecting rural agricultural communities situated in tropical and subtropical regions [1]. This disease is characterized by its huge impact on young to middle-aged populations, and notably occurs in the absence of conventional risk factors such as diabetes mellitus and hypertension [2]. The disease presents a complex and poorly understood epidemiological pattern, underscoring the urgent need for intensified research efforts to elucidate its etiology, risk factors, and effective prevention strategies. Against this backdrop, Chronic Kidney Disease of unknown etiology (CKDu) has become a pressing issue, primarily due to its unidentified causative factors. Unlike traditional CKD forms, which are typically associated with well-established risk factors such as diabetes, hypertension, and other known etiologies [3]. CKDu predominantly affects rural agricultural communities in developing nations. Its frequent clustering suggests that environmental and occupational exposures play a central role in its occurrence [4]. In Northern Yobe State, Nigeria, CKDu has become a rising public health concern, mirroring trends observed in similar settings.

However, the absence of geocoded hospital-based data and incomplete diagnostic records significantly impede accurate spatial epidemiological analyses in Nigeria. For instance, a study assessing the performance of health information systems in Ondo and Ekiti States revealed considerable gaps in data accuracy and completeness, with data accuracy reported at 70.1% in Ondo and 40.4% in Ekiti, and data completeness at 82.9% and 44.2%, respectively [5]. Similarly, a retrospective review of inpatient health records at the Federal Medical Centre, Bida, indicated that while prompt documentation was high (98.49%), the utilization of discharge summary forms was notably low (12.84%), and proper entry of patients' details stood at less than 60%, highlighting serious deficiencies [6]. These deficiencies emphasize the necessity of adopting community-based, geospatially assisted approaches to obtain more specific and contextually accurate health data for research, particularly in semi-urban settings where such data are readily lacking. The challenge is further exacerbated by the lack of geospatial information, such as accurate patient address data, which is critical for revealing spatial patterns and associations between CKDu incidence and environmental determinants [7]. This limitation obstructs effective spatial analysis and makes it difficult to investigate potential risk factors like water quality and agricultural activities, which are known to vary across different locations in Northern Yobe State.

Addressing this data gap necessitates the adoption of community-based strategies that actively involve local populations in disease surveillance. Such approaches have proven effective in enhancing data collection and reliability, as demonstrated by [8] in their research on colorectal cancer in Iowa. Engaging community members in identifying and reporting CKDu cases enables researchers to develop a more comprehensive and accurate representation of the disease's spatial distribution. Similarly, GIS-fluent approaches have been shown to improve health outcomes in low-resource settings, where access to healthcare services is often limited. For instance, successful initiatives include dengue disease surveillance in developing countries conducted by [10], as well as the containment of bovine paralytic rabies outbreaks in rural Mexico [11]. Collectively, these examples highlight the value of community engagement in addressing emerging public health challenges.

Despite these demonstrated benefits, public health interventions frequently fail to adequately prioritize high-incidence areas. In Nigeria, for example, several studies have shown that malaria control programs have historically focused on urban centers, even though higher transmission rates persist in peri-urban and rural communities [12]. Such patterns reflect broader systemic challenges in translating spatial epidemiological evidence into spatially equitable public health responses. To address these limitations, integrating community-centric approaches with Geographic Information Systems (GIS) offers a robust and effective solution. GIS has significantly advanced health research by enabling the visualization and analysis of spatial epidemiological data, thereby improving understanding of disease distribution patterns, associated risk factors, and resource allocation strategies. During the COVID-19 pandemic, for instance, GIS tools were critical in tracking infection hotspots, forecasting disease spread, and informing public health interventions globally [13]. Similarly, GIS-based malaria mapping has played a key role in identifying transmission zones and guiding targeted vector control efforts in sub-Saharan Africa [14].

Beyond infectious diseases, GIS applications in health research include mapping and modeling the spatial distribution of cardiovascular conditions and their associated risk factors [15]. GIS has also been used to analyze mosquito distributions and their environmental determinants, supporting the design of vector-borne disease control strategies and targeted public health campaigns [16, 17, 18]. In resource-limited settings, open-source GIS platforms provide cost-effective tools for collecting, managing, and analyzing spatial data [19]. These platforms enable the mapping of CKDu prevalence alongside environmental

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variables such as water quality, agricultural practices, industrial activities, and other comorbidities suspected to contribute to CKDu incidence [20, 21, 22]. Through this spatial approach, researchers can identify geographic clusters or "hotspots" of CKDu, thereby highlighting areas with significantly elevated disease burden for targeted investigation and healthcare intervention.

Against this backdrop, the present study integrates GIS technology with a community-driven data collection approach to provide a comprehensive analysis of the spatial prevalence of diagnosed CKDu in Northern Yobe State. By identifying disease hotspots, the study seeks to enhance understanding of CKDu spatial distribution patterns while also assessing the effectiveness of the applied community-centric, GIS-based methodology.

METHODOLOGY

Study Area

The study was carried out in Northern Yobe State, Nigeria, focusing specifically on Bade Local Government Area, a semi-arid region characterized by a fragile ecosystem, heavy reliance on groundwater resources, and extensive agricultural practices. The environmental landscape presents several risk factors potentially linked to the emergence and prevalence of Chronic Kidney Disease of unknown Etiology (CKDu). Notably, communities depend heavily on shallow wells, boreholes, and informal water sources, which are often susceptible to contamination by naturally occurring nephrotoxic elements such as fluoride, heavy metals (example, cadmium, arsenic, lead), and nitrates. Additionally, the widespread use of agrochemicals, including fertilizers and pesticides, in irrigation farming raises concerns about the leaching of toxic substances into groundwater supplies [23]. Combined with climatic stressors such as high temperatures and prolonged dry seasons, which can exacerbate dehydration and concentrate pollutants, these factors create an environmental backdrop conducive to renal stress and potential CKDu development [24]. Thus, the interplay between groundwater quality degradation, agricultural intensification, and climatic vulnerability forms the critical environmental context underpinning the disease risk landscape in the study area. The study area was purposively selected due to the high endemicity of rampant cases of the disease CKD [25, 26]. This region is an arid and semi-arid region known for its harsh environmental conditions.

The area is characterized by open water bodies (rivers), extreme heat waves, and intensive paddy rice agriculture and fishing activities, which serve as a primary livelihood for the local population. It is a riparian community along the river Yobe, in Northeastern Nigeria (Figure 1).

Bade Local Government Area (LGA) is strategically located in Yobe State, sharing its northern boundary with Nguru and Karasuwa LGAs and its eastern border with Jakusko LGA. The central town, Gashua, serves as both the administrative and economic hub of the region, located at approximately latitude 12°52′N and longitude 11°03′E. The town's pivotal role underscores the region's socioeconomic significance in Northern Yobe.

In Bade, groundwater serves as a primary source for domestic consumption and irrigation, yet its quality and availability pose significant public health concerns. The region's water resources are often limited and prone to contamination, raising concerns about safe drinking water access [27]. Persistent droughts and insufficient water infrastructure exacerbate these risks, creating a challenging environment for the population [28]. These conditions are suspected to contribute to the increased prevalence of chronic kidney disease of unknown etiology (CKDu) in the area [29]. Additionally, the widespread use of agrochemicals and fertilizers in farming further intensifies potential environmental health risks in the region. Many farmers in Northern Yobe heavily rely on these substances to enhance crop yields, inadvertently exposing themselves and the surrounding environment to hazardous chemicals [30]. This intensive agricultural practice is considered a contributing factor to the region's rising CKDu cases [31, 32]. Moreover, Bade experiences a semi-arid climate with distinct seasonal variations. The short rainy season occurs between April and October, while the prolonged dry season extends from November to March. The flat terrain, interspersed with undulating surfaces, features seasonal rivers that temporarily support farming and livestock activities during the wet season. Proximity to the Yobe River enhances agricultural opportunities, including fishing and irrigation. However, the semi-arid Sahelian conditions, characterized by sparse vegetation and drought-resistant flora, define the broader ecological framework [33].

The local economy primarily depends on subsistence farming and livestock rearing, both of which are significantly hindered by limited rainfall and challenging climatic conditions [34]. Gashua serves as the economic hub, linking rural communities to larger markets and supporting trade and resource distribution. The interplay between climate, terrain, and economic practices shapes the socioeconomic adaptations of Bade's population. Traditional farming and pastoral practices remain the cornerstone of livelihood strategies, deeply rooted in the region's environmental realities.

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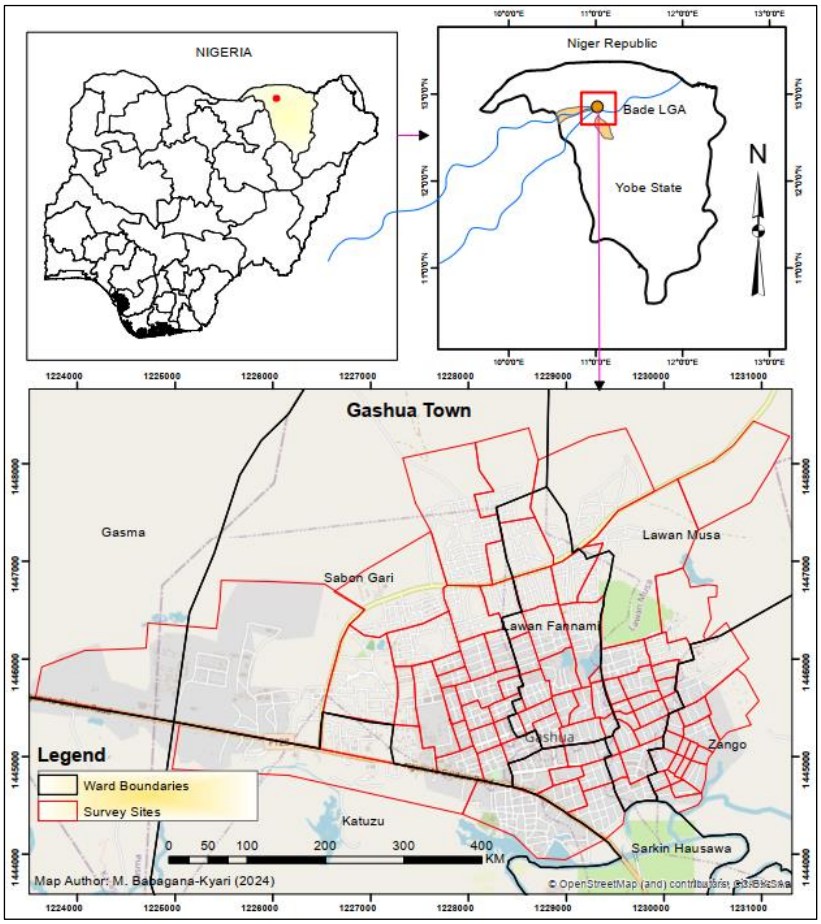

Figure 1: Study site map in Northern Yobe

Geologically, Bade lies within the Chad Basin, characterized by sedimentary formations comprising Quaternary and Tertiary deposits. These deposits, composed primarily of alluvial sands, silts, and clays, result from Ancient River and lake systems that once traversed the region. Wind-driven Aeolian processes have contributed to the formation of ancient sand dunes, particularly in the northern parts of the area [35]. These sedimentary layers support shallow aquifers, which play a critical role in sustaining water supply during the dry season, as surface water sources are scarce.

Additionally, the sedimentary geology underpins local hydrology and land-use patterns, shaping both agricultural and water resource management practices [35]. In conclusion, Bade's unique environmental, climatic, and geological features create a complex interplay of opportunities and challenges. While the region benefits from its rich natural resources and traditional adaptation strategies, issues such as water scarcity, agrochemical use, and climate variability present significant risks to public health and socioeconomic development. These factors necessitate targeted interventions to ensure sustainable management of resources and improved health outcomes for the population.

Research Approach

This study adopted a community-centric approach integrated with geospatial analysis to examine the spatial prevalence of Chronic Kidney Disease of unknown aetiology (CKDu) in Northern Yobe State, Nigeria (Figure 2). The approach was operationalized through active engagement of community leaders, health workers, and traditional authorities, who supported participant mobilization and household recruitment. Community sensitization meetings were conducted prior to data collection to explain study objectives, build trust, and obtain informed consent.

Selected community volunteers were trained to assist in administering structured surveys, identifying medically confirmed CKDu-affected households, and supporting participatory mapping using GPS-enabled tools, including the QField and KoboToolbox applications. This participatory

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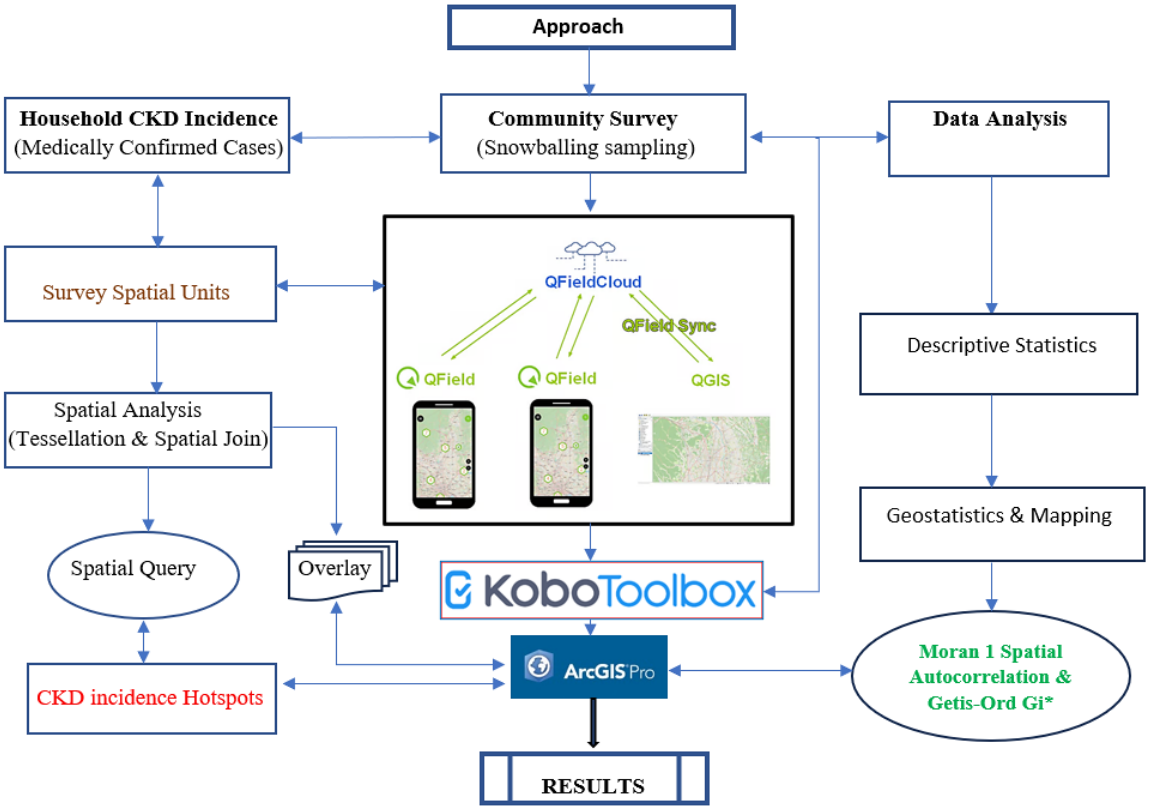

Figure 2: Methodology Flow Chart

The framework embedded local knowledge into both epidemiological and spatial data collection processes, enhancing data accuracy and contextual relevance.

Open-source GIS tools, QGIS, QField, and ArcGIS Pro 3.4, were used to map disease hotspots and analyse spatial patterns. Data were collected using electronic questionnaires deployed via the KoboToolbox platform, employing a snowball sampling method with the support of community leaders and household heads. Ethical approval was ensured through informed consent, and health-related information was collected alongside geographic coordinates for each identified CKD case. This integrated approach enabled spatial analysis of disease distribution and has been shown to be effective in low-resource, data-poor settings [36, 37].

Data Collection techniques

Data collection for this study was conducted using open-source digital tools to ensure accuracy, spatial integration, and field efficiency. Specifically, KoboToolbox was employed for administering electronic questionnaires, while QField was utilized for real-time geospatial data recording, survey area delineation, and navigation support for field workers across the study sites.

KoboToolbox enabled the structured and efficient acquisition of data on diagnosed CKDu cases. A participatory, house-to-house survey approach was adopted to identify households with confirmed cases of chronic kidney disease (CKD). Inclusion criteria were strictly based on cases verified through medical documentation; households were requested to present medical records, which were checked to ensure data reliability. The field activities were preceded by a reconnaissance survey in Lawan Fannami Ward, which served to familiarize the research team with the terrain, community dynamics, and logistics. A total of six trained field workers conducted the main survey over a period of 28 consecutive days, ensuring full spatial coverage and community engagement throughout the data collection phase.

Case Identification and Sampling Technique

Households affected by CKD/CKDu or renal failure within the study area were identified using a snowball sampling approach. This method was considered appropriate due to the absence of centralized disease registries and geocoded health

records, the exploratory nature of the study, and the social sensitivities associated with the disclosure of chronic illness. Snowball sampling facilitated case identification through trusted community referrals, thereby improving access to diagnosed cases in a low-resource and potentially stigmatized context.

Recognizing that snowball sampling is inherently non-random and may introduce selection bias, particularly the over-representation of socially connected households and under-representation of isolated cases, specific measures were implemented to enhance methodological rigor. Recruitment was initiated concurrently across multiple wards with the support of community leaders to improve spatial coverage and reduce clustering driven solely by social networks. In addition, the study area was subdivided into predefined spatial units (polygons) derived from Enumeration Areas (EAs) established under the DLI 11.3 project. These spatial units were assigned to trained fieldworkers to guide household identification, minimize duplication of case reporting, and reduce spatial misrepresentation.

Case identification relied primarily on household self-reports of diagnosed renal failure and was corroborated, where available, using hospital records and patient hand cards. Given the non-clinical and spatial epidemiological focus of the study, cases were operationally classified as CKDu when no clearly stated clinical etiology, such as diabetes, hypertension, or hereditary kidney disease, was reported. Records with missing or uncertain diagnostic information were retained for spatial prevalence mapping but excluded from etiological interpretation to minimize misclassification bias.

Although some refusals were encountered, particularly among households hesitant to disclose health information, data integrity and spatial precision were maintained through GIS-enabled field coordination. Field assistants used the QField mobile application for real-time geospatial tracking of survey activities, ensuring clear demarcation of household coverage and systematic progress monitoring. As a result, duplication and over-reporting were minimized, and comprehensive spatial coverage of the study area was achieved despite fieldwork challenges. Overall, 441 confirmed CKDu cases were recorded across 430 surveyed households. Fieldworker assignments followed coded Enumeration Areas based on the 2022 Yobe State Geographic Information Service (YOGIS) property enumeration shapefile developed for the Disbursement Linked Indicator (DLI 11.3) project, as illustrated in Figure 3.

Spatial Data Analysis

The study employed a tessellation approach to systematically divide the study area into regular, non-overlapping hexagonal spatial units. This method allowed for consistent spatial partitioning, facilitating the aggregation of CKDu cases within clearly defined boundaries for subsequent analysis. Each hexagonal grid covered an approximate area of 20,000 square meters (0.02 square kilometres or 4.9 acres), providing a uniform sampling framework across the study region. Treating each hexagon as a discrete sampling unit, the study enabled the standardized aggregation of household-level CKDu incidence data. A spatial join operation was performed to associate recorded CKDu cases with their corresponding hexagonal units, thereby enabling the systematic quantification of disease counts per standardized spatial area. This structured approach enhanced the uniformity of spatial comparisons, minimized potential sampling biases, and supported the identification of spatial clustering patterns of CKDu incidence across the study landscape (Figure 4).

To detect and evaluate the spatial patterns of CKDu prevalence, the study utilized Moran's I spatial autocorrelation as a key analytical tool. Moran's I is a statistical measure that determines whether the spatial distribution of disease cases across the study area is random, clustered, or dispersed. A positive Moran's I value signifies spatial clustering, indicating areas where cases are more concentrated, while a negative value suggests spatial dispersion, pointing to regions where cases are more spread out. Values near zero indicate random spatial patterns. For example, a Moran's I value of 0.1 implies weak but positive clustering, meaning that CKDu cases are somewhat closer to one another than would be expected by chance, though the clustering is modest. The statistical significance of Moran's I value was assessed through a permutation test involving multiple random simulations, ensuring that the observed spatial autocorrelation was not attributable to random chance. This analysis provided the foundation for understanding the broader spatial dynamics of CKDu incidence across the study area.

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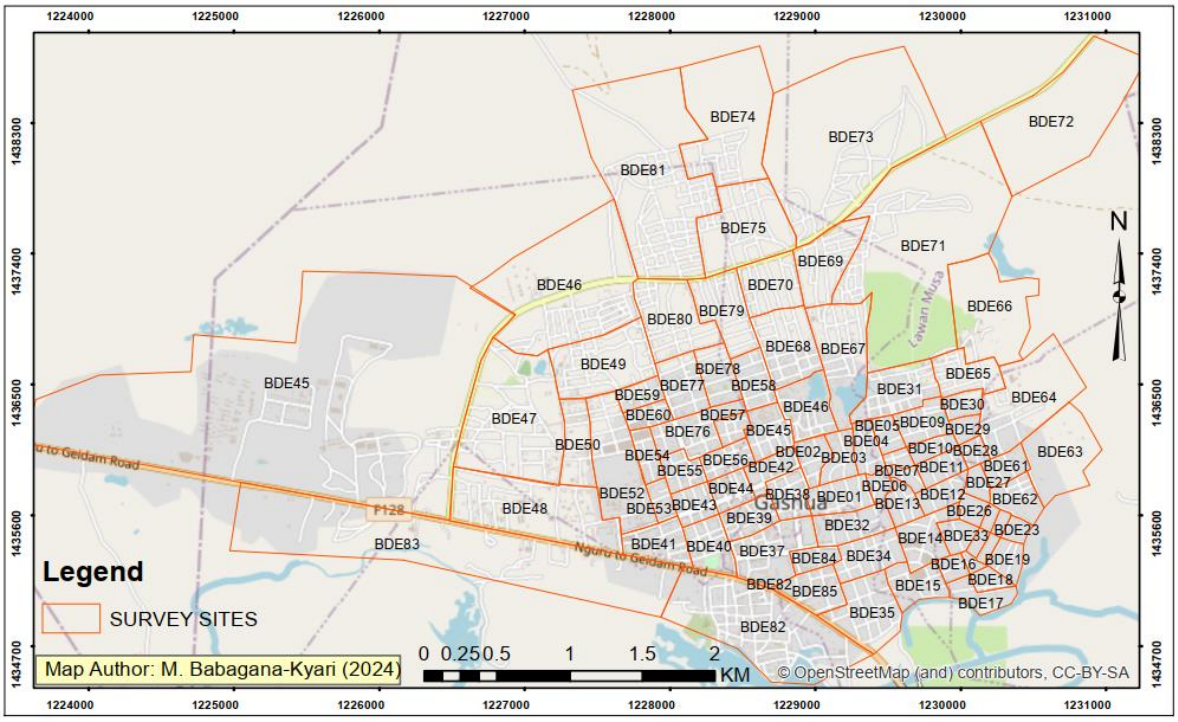

Figure 3: Survey blocks used in the Qfield App for field data collection

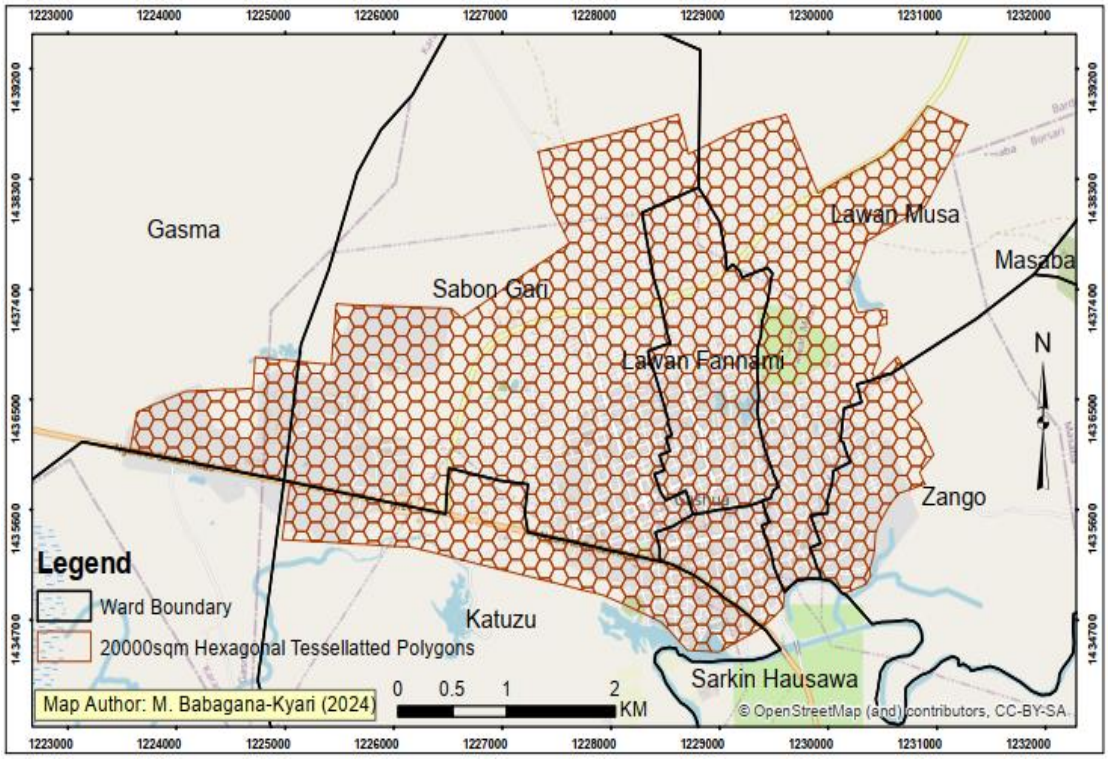

Figure 4: Tessellated polygons used for the disease incidence counts

Hotspot Analysis Using Getis-Ord Gi*

Following the spatial autocorrelation analysis, hotspot analysis was conducted using the Getis-Ord Gi* statistic. This advanced spatial technique identifies statistically significant clusters by calculating z-scores for each spatial unit. Positive z-scores indicate hotspot areas with higher-than-expected CKDu incidence, while negative z-scores denote cold spot areas with lower-than-expected incidence. The magnitude of the z-score reflects the intensity and statistical confidence of the clustering. Higher absolute z-scores correspond to more statistically significant clusters. Through this method, three distinct and statistically significant CKDu clusters were identified within the study area, categorized into high, medium, and low prevalence zones. These spatial patterns are critical for identifying zones of elevated disease risk, which may be influenced by environmental exposures, socioeconomic factors, or other local determinants. The analysis highlighted specific locations warranting further in-depth investigation to better understand the underlying causes of disease concentration.

Classification of Disease Prevalence

To facilitate meaningful interpretation of disease counts, geometric interval and standard deviation classification methods were employed. These classification schemes allowed for the stratification of CKDu prevalence into logical and analytically robust categories. By applying these methods, the spatial distribution of CKDu incidence was more clearly delineated, supporting a more intuitive understanding of disease intensity spatially across the study area.

Statistical Analysis

Descriptive statistics, including frequency distributions, percentage calculations, and graphical representations such as bar charts, pie charts, and spatial maps, were performed using IBM SPSS Software version 19.0 to analyze the socio-demographic characteristics of CKD-affected households and capture perceptions of disease aetiology. As the study adopted a descriptive epidemiological design, the analysis focused on summarizing patterns and spatial distributions rather than conducting inferential statistical tests. No formal hypothesis testing (e.g., chi-square, t-tests, or regression analyses) was carried out; however, cross-tabulations were utilized to explore variations between categories for pattern identification. This approach was consistent with the study's objective of providing a comprehensive descriptive and spatial overview of CKDu prevalence in a low-resource setting.

RESULTS AND DISCUSSION

Spatial patterns of CKD incidence distribution

Figures 5 and 6 present the spatial distribution and clustering of Chronic Kidney Disease (CKD) incidences across the study area, analysed using the Getis-Ord Gi* statistic to identify statistically significant spatial patterns. The results reveal distinct spatial clustering of reported CKD cases, with high-density hotspots prominently concentrated in the central parts of the study region. These hotspot areas represent locations with significantly elevated disease burden relative to their surroundings, indicating that CKD occurrence was spatially non-random. In contrast, cold spot areas, depicted in green, were characterized by low or negligible reported CKD incidence, suggesting fewer documented cases in these locations. Peripheral zones of the study area exhibit moderate to low incidence densities, which may reflect variations in population distribution, differences in exposure to potential risk factors, or reduced case identification in less densely settled areas. Although the observed clustering highlights clear spatial disparities in disease occurrence, the present analysis does not establish direct causal relationships between CKD incidence and specific environmental or socioeconomic determinants. Overall, the integration of spatial statistical analysis with community-derived data demonstrates the utility of GIS-based approaches for identifying disease concentration patterns in data-constrained settings. These findings support the use of spatial clustering outputs as an evidence base for guiding further epidemiological investigations and informing geographically targeted public health planning and resource allocation. Figure 7 illustrates the spatial statistics derived from Moran's I index, applied to analyze the patterns of chronic kidney disease (CKD) incidences in the study area. The results show a Moran's Index of 0.104632, with a z-score of 4.954559 and a p-value of 0.000001. Given the high z-score of 4.954559, there is less than a 1% probability that the observed clustering of CKD incidences occurred by random chance. This strong statistical evidence suggests the presence of non-random, spatially dependent factors influencing the distribution of CKD within the study area. The presence of these spatial clusters implies that the concentration of CKD incidences is not due to mere coincidence but may be driven by underlying environmental, socioeconomic, or health-related factors. Such clustering patterns highlight critical zones that warrant focused research attention, particularly groundwater quality exploration, as previous studies [30] implicated groundwater as a potential aetiology of the disease. Moreover, policymakers and health authorities can leverage these findings to prioritize environmental health surveillance and implement localized preventive strategies in high-risk zones.

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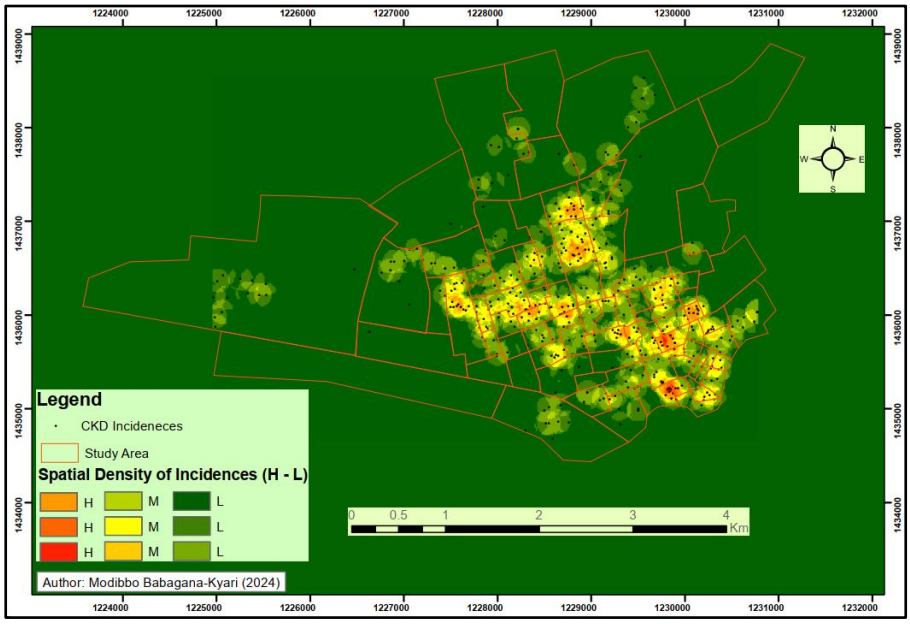

Figure 5: Spatial Distribution of CKDu Cases

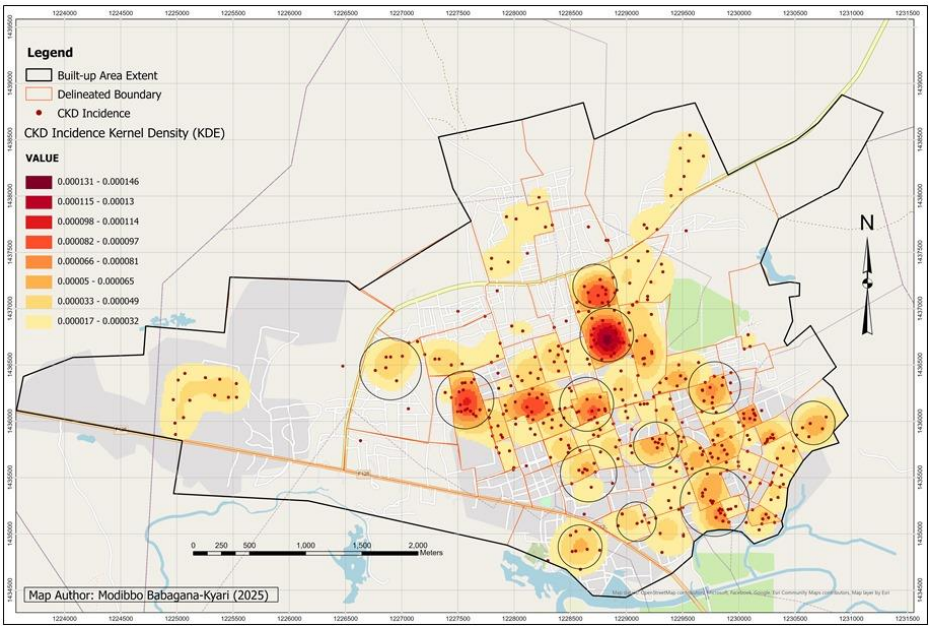

Figure 6: The identifiable hotspots of the disease in the area

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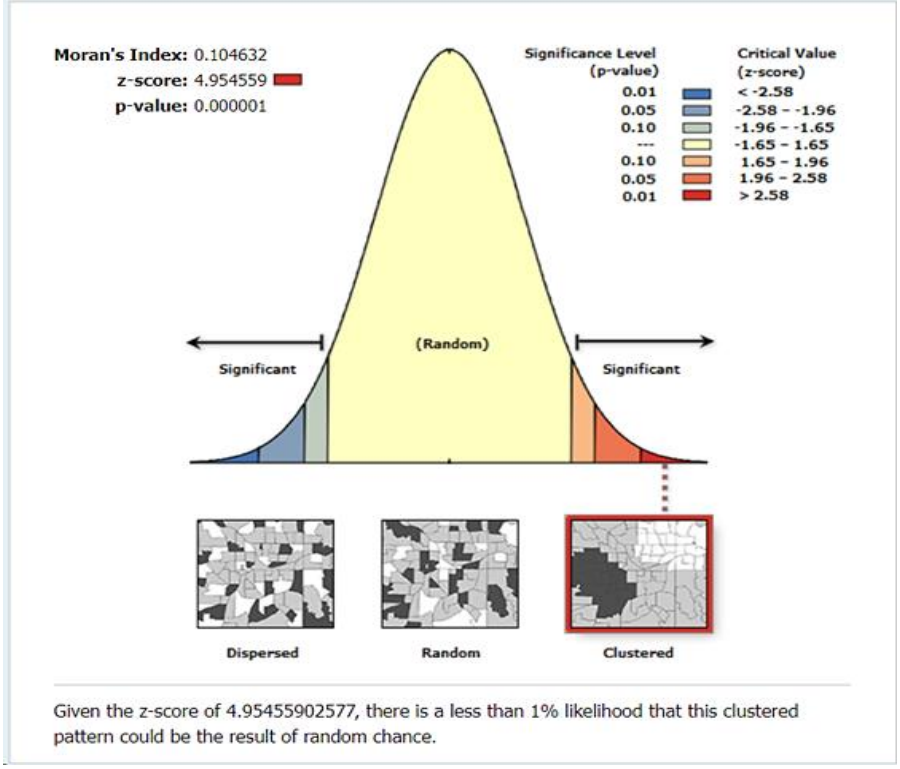

Figure 7: Spatial Statistics for incidence distribution over the area

According to Palaniyandi *et al* [37], the spatial clustering of diseases is oftentimes not merely coincidental, but rather influenced by underlying demographic, environmental, or lifestyle factors and occupational exposure factors. These factors can create conditions for disease prevalence, leading to observable patterns of clustering within specific geographic areas [38]. In the case of CKD, such clustering might be linked to localized environmental exposures, such as contaminated water sources, or specific behavioral or socioeconomic conditions, which warrant closer investigation to identify the root causes of the spatial distribution [39]. Therefore, the identified clustering pattern calls for further investigation into potential causes, such as water quality, lifestyle, or other localized risk factors that could be contributing to the uneven distribution of CKD in the study area. Exploring these factors is essential to understanding the underlying mechanisms driving the spatial concentration of CKD cases and to inform targeted public health interventions aimed at reducing the disease burden in hotspot areas.

The map in Figure 8 depicts the incidence of chronic kidney disease (CKD) across the surveyed area using hexagonal tessellation. Color coding is employed to differentiate between three levels of CKD hotspots. Dark red hexagons represent areas with the highest CKD incidence, indicating high hotspots with values ranging from 7 to 10 cases. Medium hotspots, where CKD incidence is moderate (values of 3-6), are shown in lighter red. Low-incidence regions, referred to as cold spots, are illustrated with lighter red hexagons (values of 1-2). The survey area is delineated in grey, with hotspot classification performed using the geometric interval method in ArcGIS Pro 3.2. This classification technique is tailored for skewed data distributions, producing class boundaries that grow geometrically. It is particularly effective for handling unevenly distributed data, ensuring that each class remains visually distinct, even when values cluster at one end of the scale. By employing geometric progression, this method balances class sizes, making it well-suited for datasets that exhibit exponential growth or decline patterns.

This visual representation clearly delineates the spatial distribution of CKD, enabling the identification of areas with varying disease prevalence across the study area. Previously, Kang *et al* [40] have demonstrated practically that grid-based spatial modeling can be more effective than census tract approaches for measuring spatial distribution. Spatial tessellation is a powerful analytical approach for measuring the distribution of phenomena across space. This method has been applied to various diseases, including COVID-19, Newcastle disease, and bovine tuberculosis [41, 42].

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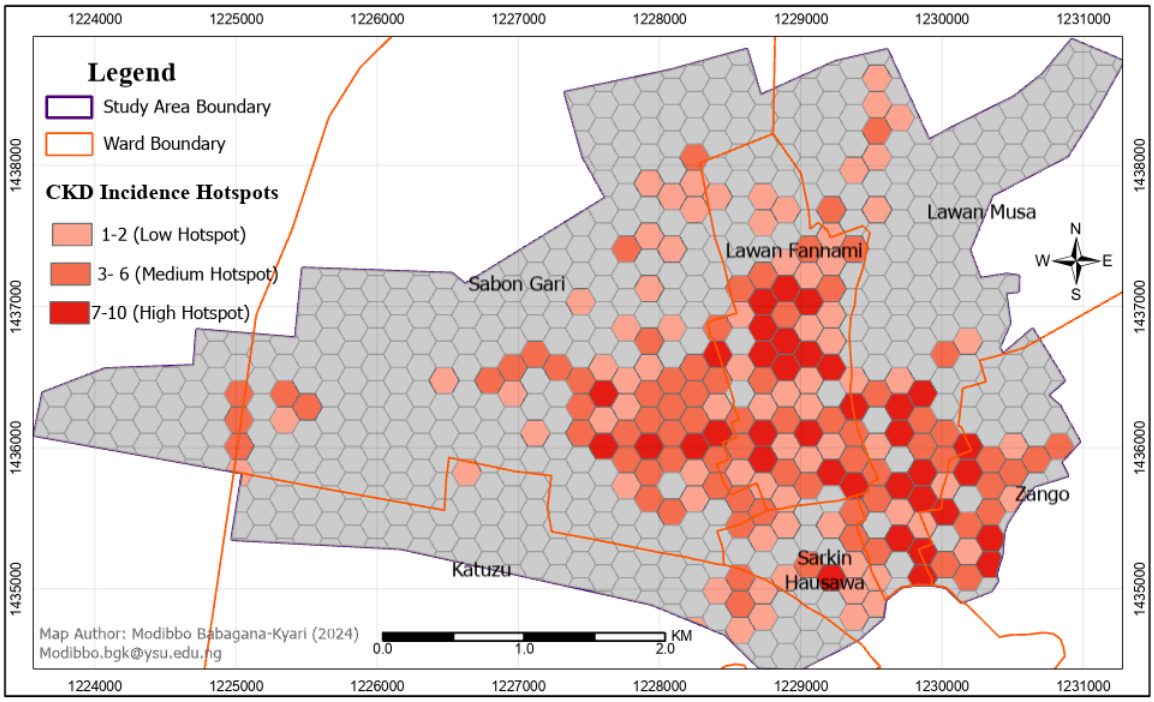

Figure 8: The three identified hotspots based on the spatial density of the incidence

Spatial Distribution Analysis of the disease hotspots

Furthermore, to explore the spatial distribution of the disease incidence in greater depth, Figure 9 provides a clear depiction of chronic kidney disease (CKD) incidence across the study area, classified using the geometric standard deviation classification method in ArcGIS Pro, 3.2, which creates classes relative to the mean value by displaying standard deviations from the mean incidence rate as presented below. From the map, it can be seen that the regions are divided into three primary hotspots based on their deviation levels: high, medium, and low incidence areas. High-incidence hotspots, with a standard deviation greater than 2.5, reflect significantly elevated CKD cases compared to the average, indicating areas requiring urgent healthcare intervention and further research. Medium-incidence areas, with a standard deviation between 1.3 and 2.3, represent moderately elevated CKD rates. This tiered visualization highlights the gradient of disease distribution across the study area. This approach assesses the effectiveness of incidence distribution across hotspots by organizing data according to deviations from the mean, creating classes based on standard deviation intervals. It is especially appropriate for datasets that closely resemble a normal distribution, as it emphasizes the degree of deviation from the mean, providing a statistical insight into data variability. However, while Freier *et al* [43] noted the limitations of this approach with skewed distributions, adjustments can be made to accommodate such data by applying transformations or alternative classification methods. These modifications can enhance the method's flexibility, allowing for more accurate representation of data with non-normal distributions, ensuring a broader applicability across various types of datasets.

Basic profiles of the CKDu victims and their household characteristics

Consent for the study participation

During the survey, participants were informed that participation was entirely voluntary and that declining would incur no penalty. The findings indicate an overwhelmingly high level of willingness to participate, with consent obtained from nearly all surveyed households and only one respondent declining participation. This near-universal consent reflects strong community acceptance of the study and can be attributed to prior community sensitization, ethical approval from the State Ministry of Health Research Ethics Committee, and institutional support from the Bade Emirate, which facilitated trust and cooperation at the community level. Overall, the high consent rate underscores the effectiveness of the community-centric approach in fostering engagement among affected households.

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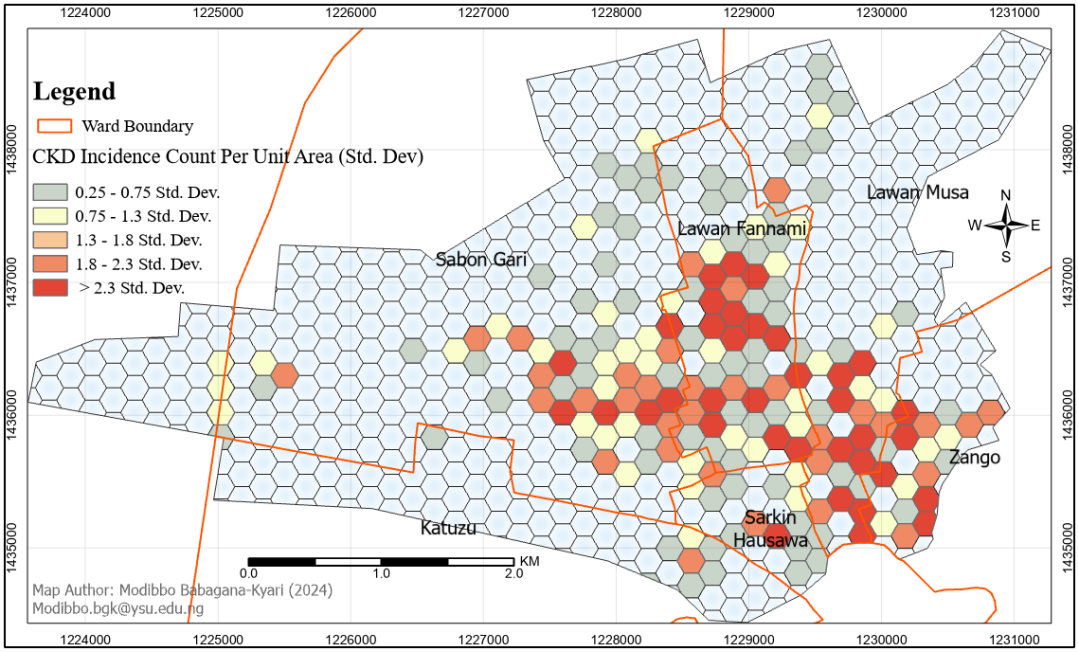

Figure 9: Depicting incidence rate distribution using standard deviation methods

Household Respondents Characteristics
This section includes details such as age, gender, and marital status of the household respondent. The respondent is typically the individual whom the field assistants encountered during the survey in the victim's household. This aspect is necessary because the CKD victims may have passed away, been hospitalized, or been physically unable to participate in an interview during the survey.

Respondent Status in household
Figure S1 provides a visual breakdown of respondent statuses. Notably, 37.44% of survey participants were household heads, while 51% were other household members. CKD patients comprised only 0.90% of respondents, suggesting a low representation likely due to mortality or morbidity. Nonetheless, household heads are considered reliable sources for healthcare information due to their comprehensive understanding of household affairs, as demonstrated by the recent study of [44].

Age Group of the respondent
Figure S2 illustrates the age distribution. It is noteworthy that the majority of the respondents fall within the 35-44 years' age group, followed closely by those in the 25-34 years' age group. The next largest group is the 45-54-year age group. Understanding the age distribution of respondents is crucial for assessing the quality of the information collected in the context of the rampant CKD incidence survey. Notably, respondents under the age of 18 years constituted less than 5% of the respondents, indicating a low representation of this age group. This also enhances the reliability of the data, as it was provided by mature adults within the surveyed households.

Educational Level of the Respondent
Table 1 illustrates the educational profile of respondents who reported the incidence of household CKD. The data shows a variety of educational levels ranging from 'High Secondary School' to 'Postgraduate degree'. The majority of respondents have a high secondary school education (51.8%, 231). This is followed by individuals with informal education (16.8%, 72) and those with some college or a bachelor's degree (15.7%, 68). Lower percentages are noted in primary school education (6.5%, 28), Islamic education (4.4%, 19), and postgraduate degrees (2.8%, 12), highlighting a diverse educational background among the surveyed individuals. The total count of respondents is 430, accounting for 100% of the data presented in the table. The insights indicate that a significant percentage of respondents were literate, suggesting they fully understood the questionnaire used. Relatively, with 51.8% having completed high secondary school and 15.7% having some college or a bachelor's degree, the majority have a solid educational foundation. Research consistently demonstrates that individuals with higher levels of education tend to have higher response rates in health surveys [44]. Additionally, the presence of respondents with postgraduate degrees (2.8%)

highlights their literacy and comprehension levels. Gender of the respondent.

Table 2 illustrates the distribution of the gender of the respondents. From the data table, it can be seen that 81.6% were male while 14.8% were female. This is because the majority of the persons who participated in the study were house heads (HH) who were Males. The gender distribution of respondents in the CKD incidence survey provides significant context for understanding the demographics of those reporting household CKD incidences.

Distribution CKD Incidence per Household

The distribution of CKD incidence per household is illustrated to show how widespread the disease is within the study area. Table 3 presents the distribution of CKD incidences from the surveyed households. The data from Table 3 reveals that the majority of households surveyed in the study area, approximately 96.37%, have experienced one incidence of Chronic Kidney Disease (CKD). A smaller percentage, 3.62%, reported two incidences, indicating that while CKD is prevalent within the community, multiple cases in the same household are relatively uncommon. The high percentage of households with single CKD cases suggests that CKD is a significant health concern, though it generally affects only one member of each household. This pattern may imply that genetic factors are not the primary cause of the disease, as multiple cases within families are rare.

To address this, interventions such as regular monitoring, educational programs, and lifestyle modifications could be implemented to help manage CKD more effectively in the community. However, early detection and regular health check-ups could also prevent the development of additional cases within the same household. However, the presence of multiple CKD cases in 2.46% of households may indicate the need for further investigation into potential genetic or environmental factors contributing to the disease. Understanding these influences could aid in developing more targeted and effective preventive measures, as highlighted by the recent study of [26] conducted across the region.

Table 1: Educational level of the respondent

Category	Frequency	Percentage (%)
High Secondary School	231	51.8
Informal Education	75	16.8
Some colleges/ Bachelor's degree	70	15.7
Primary School	29	6.5
Islamic education	18	4.0
Postgraduate degree	7	1.6
Total	**430**	**100.0**

Source: Researchers' Fieldwork (2023)

Table 2: Gender distribution of the household respondents

Category	Frequency	Percentage (%)
Male	364	81.6
Female	66	14.8
Total	**430**	**100.0**

Source: Researchers' Fieldwork (2023)

Table 3: Number of CKD incidence in households in the study Area

Options	Frequency	Percentage (%)
1 incidence	425	96.37
2 incidences	16	3.62
Total	**441**	**100.0**

Source: Researchers' Fieldwork (2023)

Household Size

The size of the household indicates the density of persons within the household. Table 4 presents the household Size, and it can be seen from the table that the category of households based on their sizes is in frequency and percentage. The largest category is "6-10 persons Household," representing 42.4% of the total surveyed households. This is followed by the "3-5 Person Household" category, which accounts for 21.7%, the "More than 10-person Household" category with 77 households representing 17.3%, the "1-Person Household" category with 53 households (11.9%), and the "2-person Household" category with 14 households (3.1%). In total, 430 households were surveyed. Thus, the data highlights the distribution of household sizes in the study area, which is crucial for understanding the context in which CKD incidence is being examined. The data suggest that the disease predominantly affects households of both larger and smaller sizes, and this household size may impact the management of CKD within the family due to the significant financial burden it imposes on them.

Victims' household income

The section presents the distribution of incomes for the surveyed households. The histogram in Figure S3 represents the income distribution of households for chronic kidney disease (CKD) victims in CKD-affected areas. The Y-axis represents the frequency of the responses, while the X-axis represents the household incomes. The data is categorized into different income ranges, and the frequency of households falling into each income range is displayed. Additionally, the percentage of households in each category is shown on the bars**.**

The findings indicate that the majority of CKD-affected households fall within low- to moderate-income categories, with over 75% earning less than ₦50,000 monthly. The most common income range was ₦20,000-₦50,000, accounting for approximately half of the surveyed households, while higher-income groups (₦100,000–₦200,000) were minimally represented. This distribution suggests that CKD prevalence is concentrated among economically disadvantaged households in the study area.

The observed income disparity may reflect differences in lifestyle and access to safe water sources. Higher-income households are more likely to invest in private boreholes or purchase bottled water, potentially reducing exposure to contaminated water—one of the suspected etiological factors of CKD. In contrast, lower-income households often depend on communal or untreated water sources, increasing vulnerability to waterborne contaminants. Empirical studies support this association, identifying low socioeconomic status as a key determinant of CKD/CKDu risk due to limited access to clean water, healthcare, and adequate nutrition [45, 46]. Additionally, poor water quality, particularly groundwater contamination by heavy metals and toxins, has been linked to elevated CKD prevalence [41]. Overall, the results highlight the economic vulnerability of CKD-affected households and its implications for health risk exposure and access to care.

Medical Confirmation of Household CKD Incidence

As shown in Table 5, the vast majority of reported CKD cases were medically confirmed, with 98.1% (433 cases) validated by healthcare facilities, while only 1.8% (8 cases) lacked medical confirmation. Several households also presented medical reports during the survey, further supporting the credibility of the data.

Table 4: Surveyed Household Size

Responses	Frequency	Percentage (%)
6 -10 persons Household	189	42.4
3- 5 Persons Household	97	21.7
2 - person household	14	3.1
1- Person Household	53	11.9
More than 10-person Household	77	17.3
Total	**430**	**100.0**

Source: Researchers' Fieldwork (2023)

Table 5: Surveyed Household CKD Incidence

Response	Frequency	Percentage (%)
Yes	433	98.1
No	8	1.8
Total	**441**	**100.0**

Source: Researchers' Fieldwork (2023)

The high rate of medical confirmation indicates a strong level of diagnostic reliability among the surveyed cases, reducing concerns about underreporting or misidentification. This finding aligns with existing evidence emphasizing the importance of clinically verified diagnoses in accurately assessing CKD burden, particularly in low- and middle-income settings where under-diagnosis is common [43, 44]. Overall, the high verification rate strengthens confidence in the reported CKD prevalence and supports the robustness of the study's findings.

Family History of CKD Victims

As presented in Table 6, the majority of households (73.3%; 343 out of 441) reported no known family history of CKD, while 18.2% (81 households) confirmed a family history. A small proportion of respondents (4.9%; 17 households) were uncertain and indicated that they could not recall.

The predominance of households without a known family history suggests that CKD occurrence in the study area may not be primarily driven by genetic factors but could instead reflect the endemic nature of the disease within the community. The widespread distribution of cases across unrelated households supports the plausibility of environmental or regional influences contributing to CKD prevalence. This interpretation aligns with previous studies that associate elevated CKD occurrence with local environmental conditions rather than hereditary predisposition [26]. Overall, the findings underscore the need for further investigation into non-genetic risk factors underlying CKD in the study area.

CKD victim's status

Table 7 presents data on the status of CKD (Chronic Kidney Disease) victims from the surveyed households. The majority of the surveyed CKD victims, 75.96%, have died, indicating a high mortality rate among the respondents. In contrast, 22.9% of the surveyed patients are still alive, providing a perspective on the survival rate. Additionally, a small proportion of the surveyed individuals (1.13%) opted not to disclose their health status. While numerically minor, this non-disclosure may reflect underlying issues such as social stigma, fear of discrimination, or cultural sensitivities associated with chronic disease reporting. Such reluctance to share health information could impede accurate disease surveillance among the population. Recognizing and addressing these barriers is crucial for improving trust, data quality, and the inclusiveness of public health strategies aimed at CKD prevention and management, as well as other diseases. The survival rate among the respondents is relatively low, with only 22.9% of the patients still alive. This statistic provides a stark contrast to the high mortality rate and emphasizes the life-threatening nature of CKD. Additionally, a small portion of the surveyed individuals (1.13%) chose not to disclose their status, which, while a minor part of the dataset, indicates some level of privacy concern or stigma associated with the disease.

Kidney Disease Type among Victims

Table 8 illustrates the prevalence and types of kidney diseases among the participants. The data table below categorizes the diseases into six types: Glomerulonephritis, Kidney Stone/Kidney Cyst, Diabetic Kidney Disease, Kidney Infection, Hypertensive Nephropathy, and Complicated Cases. Additionally, there is a category for participants who had no clear idea about their type of kidney disease. This classification was obtained in relevant literature, hence was adopted and used.

Table 6: Households with a known Family History of CKD

Responses	Frequency	Percentage (%)
No	343	73.3
Yes	81	18.2
Can't recall	17	4.9
Total	**441**	**100.0**

Table 7: Statuses of CKD victims surveyed from the household

Patient Status	Frequency	Percentage (%)
Died	335	75.96
Alive	101	22.9
Preferred Not Say	5	1.13
Total	**441**	**100**

Source: Researchers' Fieldwork (2023)

Table 8: Disease type among surveyed victims in the area

Disease Types	Frequency	Percentage (%)
a) Complicated case	135	30.61
b) Hypertensive CKD	109	24.71
c) Glomerunephritis	5	1.13
d) Kidney infection	61	13.83
e) Kidney Stone/Cyst	7	1.58
f) Diabetic Kidney Disease	19	4.30
g) No Idea about the disease Type	105	23.80
Total	**441**	**100.00**

Source: Researchers' Fieldwork (2023)

Table 8 shows that *complicated cases* constituted the largest proportion of reported CKD conditions (30.27%), while a substantial share of participants (24.66%) were uncertain about their specific disease type. In this study, *complicated cases* refer to individuals who were unable to clearly describe their condition or who were diagnosed with CKD after presenting with multiple coexisting health problems. This pattern suggests limited engagement in routine health monitoring within the community, leading to delayed diagnosis and compounded health conditions.

Hypertensive CKD accounted for 24.44% of cases, indicating a notable association between hypertension and kidney disease. Kidney infections represented 13.68% of reported conditions, while diabetic kidney disease was less common (4.26%). Other conditions, including kidney stones or cysts (1.57%) and glomerulonephritis (1.12%), were relatively rare. Overall, the high proportion of participants who were unsure of their disease type supports the operational classification of many cases as CKD of unknown etiology (CKDu). This diagnostic uncertainty underscores limitations in healthcare access and diagnostic capacity and highlights the need for improved community health education, early screening, and accessible diagnostic services to enable timely detection and management of chronic kidney disease.

CKD Victim's Medical History

Table 9 provides a breakdown of the medical history of Chronic Kidney Disease (CKD) patients, categorizing their conditions with detailed counts and percentages. Hypertension emerges as the most prevalent condition, affecting 38.10% of the patients, while diabetes follows, present in 7.94% of cases. Combined conditions are also notable: 5.00% of patients reported both diabetes and hypertension, and 2.72% had both hypertension and diabetes, highlighting the frequent overlap of these risk factors in CKD cases. Other less common conditions include hepatitis (2.04%) and urinary tract infections (1.59%).

Notably, 41.89% of patients reported having "No Idea" about their medical history, indicating a lack of awareness or access to proper healthcare diagnostics, a common issue in CKD studies in resource-limited settings [43]. A small percentage (0.68%) preferred not to disclose their medical history. For instance, a study by [44] points to hypertension and diabetes as leading contributors to traditional CKD development, stressing the need for targeted interventions to manage these comorbidities in populations at risk. However, it remains unclear whether hypertension preceded or followed the CKD diagnosis, which is consistent with studies highlighting the bidirectional relationship between the two conditions [43].

Table 9: Medical History of CKD victims

History category	Frequency	Percentage (%)
1. Hypertension	168	38.10%
2. Diabetes	35	7.94%
3. Diabetes & Hypertension	22	5.00%
4. Hypertension & Diabetes	12	2.72%
5. Hepatitis	9	2.04%
6. Urinary Tract Infection (UTI)	7	1.59%
7. I have no Idea	185	41.89%
8. Preferred Not Say	3	0.68%
Total	**441**	**100.00**

Source: Researchers' Fieldwork (2023)

Victims' household perspectives on suspected risk factors

Figure S4 presents community perceptions of suspected CKD risk factors from the perspective of victims' households. Contaminated water quality was the most frequently cited factor (30.8%), followed by perceptions attributed to the "Act of God" (20.5%) and witchcraft (8.6%). Other commonly mentioned factors included dehydration due to heat stress, abuse of analgesic drugs, diabetes prevalence, frequent use of herbal medication, and genetic factors, each accounting for approximately 6.8% of responses. Less frequently cited causes included consumption of vegetables grown locally (5.2%), lack of knowledge about risk factors (3.0%), and agricultural-related exposures such as food crops grown in the area (1.5%), agrochemical use (1.4%), and rice cultivation (0.5%).

The prominence of contaminated water quality highlights strong community concern regarding environmental exposures, although direct water quality assessment was not included in this study. This underscores the need for future research to prioritize water quality testing, particularly within identified high-incidence hotspots, to validate community perceptions and clarify potential etiological pathways. Similar associations between contaminated groundwater, particularly heavy metals such as cadmium and arsenic, and CKDu have been reported in studies from Sri Lanka and India [45]. The attribution of CKD to supernatural causes, such as the "Act of God" and witchcraft, reflects prevailing cultural interpretations in settings where biomedical understanding may be limited.

Furthermore, the notable reporting of heat stress–related dehydration and analgesic drug misuse aligns with global evidence linking CKDu to prolonged heat exposure and frequent use of non-steroidal anti-inflammatory drugs, both of which can exacerbate renal damage. Although agricultural factors were less frequently perceived by respondents, existing literature documents agrochemical exposure as a potential contributor to CKDu in farming communities. Overall, these findings illustrate the coexistence of environmental, behavioral, and cultural explanations of CKD within the community, reinforcing the need for integrated, multidisciplinary approaches to risk-factor investigation and intervention.

Implications for Public Health Interventions

The identification of CKDu hotspots using the Open-source GIS technology with a community-centric approach offers critical insights, particularly the spatial distribution of the disease. Areas identified as hotspots should be prioritized for further investigation and intervention. Public health strategies should focus on improving water treatment infrastructure, regulating the use of agrochemicals, and increasing community awareness of CKDu risk factors, as previous studies implicated water contamination as a driver of the disease in the area. Additionally, the integration of GIS with community-centric data collection can be used to monitor CKDu incidence over time, providing valuable information for early intervention and prevention efforts as well as insights for risk factors exploration.

Critical Reflection

The integration of community-centric approaches with open-source GIS technologies represents a key strength of this study, enhancing community engagement, awareness of CKDu, and access to spatial health monitoring tools. The use of hotspot mapping effectively identified high-risk areas, providing practical insights to support targeted public health interventions in data-constrained settings.

However, the study also has limitations related to data quality and case identification. Information was often provided by household members on behalf of affected individuals, many of whom were deceased or medically incapacitated, resulting in reliance on morbidity and mortality data and potential reporting bias. While this may limit clinical precision, the focus on medically confirmed cases supported by hospital records in most households helped strengthen data reliability and mitigate uncertainties associated with second-hand reporting.

The study does not fully capture the complex, multifactorial etiology of CKDu, which likely involves interactions among environmental, occupational, lifestyle, and genetic factors. Future research should therefore adopt a multidisciplinary approach involving environmental chemists, nephrologists, toxicologists, epidemiologists, and public health professionals to enable a more comprehensive understanding and inform evidence-based interventions. Additionally, the context-specific nature of the findings may limit generalizability beyond the study area. Replicating this approach in ecologically and demographically diverse regions would help validate observed spatial patterns and assess the broader applicability of identified risk factors. Overall, the study contributes methodologically to understanding CKDu spatial prevalence in Northern Yobe State and provides a foundation for future research and targeted interventions.

Future Research and Policy Recommendations

Further research is needed to investigate the specific environmental mechanisms contributing to CKD of unknown etiology (CKDu) in Northern Yobe State, with particular emphasis on groundwater contamination, chemical exposures, and the disease spatial prevalence. Groundwater quality assessments and geospatial analyses are essential for identifying environmental risk factors linked to CKDu in the region. To strengthen exposure-disease associations, future studies should also incorporate biomedical monitoring, such as blood or urine sample examination for a subset of the

population, providing direct evidence of internal exposure to nephrotoxic agents, particularly in groundwater and food resources, as their major pathways.

Equally, policymakers should prioritize investment in safe water infrastructure, particularly in high-risk communities. Key actors well-positioned to drive this initiative include the Yobe State Government, the Federal Ministry of Water Resources, and WASH sector programs operating at both national and sub-national levels. Additionally, efforts to enhance hospital record-keeping and tracking systems, particularly through geocoding of hospital data, would improve the ability to monitor CKDu and other environmentally induced diseases. This would not only benefit the affected communities in Northern Yobe State but the Country as a whole.

CONCLUSION

This study demonstrates that Chronic Kidney Disease of unknown etiology (CKDu) in Northern Yobe State exhibits clear spatial clustering, with identifiable hotspots that reflect non-random disease distribution. The identification of these hotspots has direct public health relevance, as it provides an evidence base for prioritizing high-burden communities for targeted surveillance, environmental investigations, and preventive interventions. In settings where health resources are limited, such spatial intelligence is critical for guiding efficient allocation of healthcare services, risk communication, and environmental health assessments, particularly in relation to suspected exposures such as unsafe water sources and heat-related stress.

The findings further show that a community-driven, GIS-based approach can effectively generate spatially explicit disease evidence in data-poor contexts where formal disease registries and geocoded health records are unavailable. By integrating participatory data collection with open-source geospatial tools, this methodology enables early identification of high-risk areas and supports context-specific public health decision-making. Importantly, the approach is scalable and adaptable, making it suitable for application in other resource-limited regions facing similar challenges in disease surveillance and environmental health assessment. Overall, this study provides a practical framework for leveraging community participation and spatial analysis to strengthen CKDu surveillance, inform policy development, and guide targeted interventions in vulnerable populations.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in this work.

ETHICAL APPROVAL

Prior to the study, ethical approval was duly sought and granted by the Yobe State Ministry of Health's Research Ethics Committee, ensuring adherence to ethical guidelines for research involving human subjects. Additionally, informed consent was sought from all participants, with the assurance that their personal data would be anonymized and used strictly for research purposes, and all consented. The involvement of neighborhood leaders facilitated trust-building within the community, which was essential for the accurate reporting of CKDu cases.

AUTHORS' CONTRIBUTION

M. Babagana-Kyari participated in the design of the study, data collection, GIS-based analysis, manuscript preparation, and final review. N. A Yaro provided guidance on the study design, supervised data analysis, reviewed and edited the manuscript, and contributed to the interpretation of findings. Similarly, K. M Yakasai participated in the supervision of data collection, provided critical input on methodology, reviewed the manuscript, and contributed to the final revisions.

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The Effect of Storage on the Proximate, Mineral Composition, and Mycoflora of Sundried Pigeon Pea (*Cajanus cajan*) Seeds

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ABSTRACT
This study investigates the impact of a twenty-week storage period on the proximate composition, mineral content, and mycoflora of sun-dried pigeon pea (*Cajanus cajan*) seeds. The storage conditions were observed to promote fungal contamination, leading to qualitative and quantitative changes in the seeds' nutritional and mineral composition. Fungal species isolated included *Aspergillus niger, A. fumigatus, A. flavus, A. oryzae, Fusarium oxysporum, F. solani, F. moniliforme, Rhizopus stolonifer, Alternaria alternata, and Penicillium notatum*. Proximate analysis (g/100g) showed significant increases in moisture content (9.86 to 13.13), ash (2.60 to 3.15), fat (1.25 to 3.17), and carbohydrate (0.22 to 10.56). However, fiber (7.75 to 4.61) and crude protein (76.74 to 68.83) content decreased, suggesting deterioration in the seeds' quality over time. Mineral analysis revealed reductions in sodium (3.49 to 2.47), potassium (21.26 to 15.03), calcium (6.02 to 3.43), magnesium (11.75 to 9.27), zinc (0.09 to 0.05), iron (0.28 to 0.21), copper (0.07 to 0.02), manganese (0.08 to 0.05), and phosphorus (19.02 to 12.03). The findings indicate that prolonged storage at room temperature facilitates mycoflora proliferation, leading to degradation in nutritional and mineral composition. This has significant implications for food security, as pigeon pea is a key protein source in many regions. The study underscores the importance of adopting improved storage techniques to minimize fungal contamination and nutrient loss, thereby enhancing the economic and nutritional value of stored pigeon pea seeds.

Keywords: Pigeon pea; *Cajanus cajan;* Storage effects; Proximate composition; Mineral composition; Mycoflora

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INTRODUCTION

Cajanus cajan (L.) Millsp., commonly known as pigeon pea, is a leguminous plant belonging to the family Leguminosae (Fabaceae). It is widely known as red gram in English and Arhar or Tur dhal in India [1]. Its seeds vary in size, shape, and color, typically round or oval, ranging from white and greyish to red and brown, with a small white hilum. Production of *Cajanus cajan* in Africa is estimated to account for about 9.3% of global production, which is comparatively small next to the 77.6% contribution from India [2]. The crop is cultivated throughout the tropics and subtropics [3,4] and represents approximately 5% of total world legume production [5], with India remaining the dominant producer [6]. Globally, pigeon pea is cultivated on about 4.67 million hectares, of which 3.30 million hectares are in India alone. In Asia, other significant producers include Myanmar (570,000 ha), China (150,000 ha), and Nepal (20,988 ha) are also important pigeon pea-producing countries. In Africa, significant production occurs in Tanzania, Kenya, Malawi, Uganda, and Mozambique [7]. India remains the world's largest producer and consumer of pigeon pea [8]. In Nigeria, pigeon pea is produced in considerable quantities, particularly in the southeastern, southern, and central regions of the country [8]. Compared with other grain legumes, pigeon pea ranks only sixth in area and production [9,10]. It contains protein (23.77%), Fat (1.1%), and crude fibre (7.49%). Its seed consists of 85% cotyledons, 14% seed coat, and 1% embryo. Cotyledons are rich in carbohydrates (66.7%), while a major proportion (about 50%) of seed protein is located in the embryo. It has a good amount of cysteine and methionine [11]. It provides iron, sulphur, calcium, potassium, manganese, thiamine, niacin, and riboflavin. [7] It is also known to contain genistein and daidzein [3]. Despite its nutritional and economic importance, post-harvest storage poses a major challenge to the quality and safety of pigeon pea and other legumes. During storage, seeds are highly susceptible to microbial contamination, especially by storage fungi such as *Aspergillus, Fusarium, Penicillium, and Rhizopus* species. These spoilage organisms cause biochemical deterioration, leading to loss of nutritional value, seed discoloration, rancidity, and off-flavors [12–14]. More importantly, some of these fungi produce mycotoxins, such as aflatoxins and fumonisins, which have been linked to serious health issues, including hepatotoxicity, nephrotoxicity, immunosuppression, and carcinogenesis in humans and animals [15,16]. Previous studies on other legumes such as cowpea (*Vigna unguiculata*), soybean (*Glycine max*), and groundnut (*Arachis hypogaea*) have documented similar patterns of fungal invasion and nutrient degradation during prolonged storage [17–19]. However, there is limited information on the mycoflora associated with sun-dried pigeon pea during storage, particularly under tropical conditions where temperature and humidity promote rapid fungal proliferation. Therefore, this study was designed to evaluate the changes in proximate composition, mineral content, and mycoflora of sun-dried pigeon pea (*C. cajan*) stored for 20 weeks. The findings aim to provide insight into the nutritional and safety implications of long-term storage and to contribute to strategies for maintaining the quality of pigeon pea during storage.

METHODOLOGY

Collection of Samples

The seeds of *Cajanus cajan* were obtained from Oja Oba market in Ado-Ekiti, Ekiti State, Nigeria. The seeds were sundried for one week. The dried pigeon pea was further stored in an insect-free container, carefully labelled, and kept in the well-ventilated laboratory for 20 weeks in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. The seeds were examined monthly for changes in mycoflora, minerals, and nutrient composition during storage.

Proximate Analysis

A sample of the stored pigeon pea was analysed using the AOAC [20] standard procedures for ash, crude fibre, moisture content, Fat, protein, and carbohydrates. All determinations were in duplicates.

Mineral Analysis

The AOAC standard method [20] was used. The minerals were analyzed from a solution obtained by first dry-washing, as follows: about 1.5 g of the flour sample was placed in a petri dish and heated gently on a Bunsen burner in a fume cupboard until the charred mass ceased to emit smoke. It was transferred to a muffle furnace at 550 °C. Heating was continued until all the carbon was burnt away. The dish and ash were transferred to a desiccator to cool, after which 0.1 M HNO^3 solution (10 mL) was added to the crucible to break up the ash. It was then filtered through acid-washed No. 43 Whatmann filter paper into 100 mL with the same dilute acid solution. Mineral concentrations (Ca, Fe, P) were determined by flame Atomic Absorption Spectrophotometry using a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc., East Norwalk, Connecticut, U.S.A). Hollow cathode lamps specific for each element were used, and an air–acetylene flame served as the energy source. Calibration curves were prepared using certified standard solutions of each metal, and the sample concentrations (mg/100 g) were calculated from the calibration curves based on their absorbance values [20].

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Isolation of Fungi from Stored Sundried Pigeon Pea (*Cajanus canjan*)
The mycoflora associated with *Cajanus cajan* during storage were isolated using three methods: direct plating, dilution plate, and washing.

Direct Plating Method
Sun-dried seeds of *Cajanus cajan* were randomly selected and surface-sterilized individually with 70% ethanol for 1 minute, followed by 2 rinses with sterile distilled water to remove residual ethanol. The seeds were then aseptically plated onto potato dextrose agar (PDA) plates using a sterile spatula and incubated at room temperature (25 ± 2°C) for 5–7 days. Emerging fungal colonies were subcultured onto fresh PDA plates to obtain pure cultures through successive hyphal tip transfers. The purified cultures were examined microscopically for morphological features, including hyphae, conidia, and fruiting bodies, to identify the common fungi present [21].

The emerging fungal colonies were subcultured onto fresh PDA plates to obtain pure cultures by successive hyphal tip transfers. The purified cultures were examined microscopically for morphological features, including hyphae, conidia, and fruiting bodies, to identify the common fungi present [22].

Dilution Plate Method
The dilution plate method described by Fagbohun and Ogundahunsi [23] was employed. One gram of the stored *Cajanus cajan* sample was aseptically placed in a test tube containing 9 ml of sterile distilled water and thoroughly shaken to obtain a uniform suspension. One milliliter of this mixture was serially diluted in sterile distilled water to achieve the desired dilution levels. Aliquots of milliliter each from the 10^{-2} and 10^{-3} dilutions were introduced into sterile molten PDA plates supplemented with 100 µg/ml chloramphenicol to inhibit bacterial growth. The plates were gently swirled for uniform mixing, allowed to solidify, and incubated at ambient temperature (25 ± 2°C) for 5 to 7 days. Emerging fungal colonies were observed for morphological features, including fruiting bodies and hyphal characteristics. Hyphal tips of each distinct fungus were transferred successively onto fresh PDA plates until pure cultures were obtained.

Washing Method
The washing method described by Fagbohun [22] was employed. One gram of *Cajanus cajan* seeds was weighed using a digital weighing balance and dispensed into a test tube containing 9 ml of sterile distilled water. The mixture was shaken thoroughly for about 2 minutes to dislodge surface contaminants and fungal spores. Using a sterile pipette, 1 milliliter of the resulting suspension was aseptically introduced into sterile Petri dishes containing molten PDA. The suspension was evenly spread on the agar surface using a sterile glass spreader to ensure uniform distribution of spores. The plates were allowed to solidify and then incubated at 28°C for 5 to 7 days. After incubation, the plates were examined for visible fungal growth. Distinct colonies were subcultured onto fresh PDA plates until pure cultures were obtained for further identification.

Identification of Mycoflora
Pure cultures of each fungus isolated from *Cajanus cajan* seeds were prepared on nutrient media, and slides were made for microscopic observation. Cultural and morphological characteristics of each isolate, including colony colour, texture, hyphae septation, spore structure (sporangia/conidia), spore arrangement (single or in chains), spore shape, and size, were examined and recorded as identification criteria [24,25]. The isolates were further subcultured to obtain pure strains, and detailed macroscopic and microscopic features (e.g., conidiophore structure, conidial morphology) were used to assign fungal genera and/or species based on contemporary identification schemes [26,27].

Needle Mount Preparation Method
Fragments from the sporulating surface of pure fungal cultures (taken from mid colony to colony edge) were gently teased in a drop of 70% ethanol on a sterile glass slide using a sterile needle. A drop of lactophenol cotton blue stain was added, a coverslip applied, and the preparation examined under 10× and 40× objective lenses [28].

Slide Culture Technique
From a plate approximately 2 mm deep, a 1 cm2 PDA was cut and placed on a sterile glass slide. Each isolated fungus was inoculated into the four vertical sides using a sterile needle. A sterile coverslip was placed on it, overlapping the medium on all sides. The preparation was placed on a suitable support in a petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. The medium was removed, and the fungus adhering to both coverslip and slide was examined [28]. A drop of alcohol was added, followed by a drop of lactophenol blue, and the preparation was covered and examined under the microscope's low-power objective.

Statistical Analysis
For each sample, the overall Mean, standard deviation (SD), and standard error of the Mean (SEM) were calculated. All analyses were performed in triplicate, and results are expressed as mean ± SD. Data from proximate, mineral, and mycofloral analyses were processed using

Microsoft Excel (version 2019). To compare differences in mean values across storage period treatments, a one-way analysis of variance (ANOVA) was applied, followed by post hoc comparisons of means using a multiple-comparison test. Differences were considered statistically significant at $p < 0.05$, and mean values bearing different superscript letters within the same column or row were considered significantly different at the 5% level [29].

DISCCUSSION

The fungi isolated from stored sun-dried *Cajanus cajan* seeds using the direct plating, washing, and dilution methods are summarized in Tables 1–3. The results revealed a progressive increase in the diversity and frequency of fungal species with increasing storage duration. At the initial stage of storage, only a few fungal species, such as *Aspergillus niger*, *A. fumigatus*, and *A. flavus*, were isolated; however, by the 20th week, ten fungal species belonging to five genera were identified across the three methods. The summary of the results of fungi isolated from stored sundried pigeon pea (*Cajanus cajan*) seeds is shown in Figure 1. Ten (10) fungi comprising five genera were isolated: *Rhizopus* sp., *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., and *Alternaria* sp. This finding is in agreement with the report of Chaudhari et al. [30], who reported the presence of *Aspergillus niger*, *A. flavus, Fusarium oxysporum, F. moniliforme, F. udum, Drechslera* sp., *Curvularia lunata*, *Rhizoctonia* sp., and *Alternaria alternata* in pigeon pea (*Cajanus cajan*) seeds. A similar observation was reported by Ingle et al. [31], who isolated multiple fungal species, including *Alternaria alternata, Aspergillus flavus, A. niger, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Fusarium oxysporum, F. moniliforme, F. roseum, Pythium sp., Rhizoctonia solani, Rhizopus stolonifer, Botrytis cinerea, Macrophomina phaseolina, Penicillium notatum, and Phytophthora cinnamomi* from pigeon pea seeds using agar plate and blotter methods. It was noted that while most fungi were recovered by all methods, *Alternaria alternata* was not isolated using the direct plating method. The predominant fungi belonged to the genus *Aspergillus*, which are commonly classified as storage fungi capable of growth at lower seed moisture content [32]. Among *Aspergillus* species, A. *flavus* was the most frequently isolated. Similar results have been reported in other legume and groundnut studies [33–35]. Several studies have shown that stored legumes and cereal grains are highly susceptible to *Aspergillus flavus* colonization, with fungal loads often increasing as storage duration progresses [36]. This contrasts with the findings of Amsalu et al. [37], who reported a decline in fungal populations during storage of faba beans. Most isolated fungi are known to be surface contaminants of seeds and contribute to decay during storage. Fungi recovered via washing methods are typically surface colonizers, while those recovered by any method may include both field and storage fungi [38]. Stored products remain vulnerable to microbial attack under favourable conditions such as moderate temperatures and high humidity [39]. These fungi also produce mycotoxins as secondary metabolites during growth, harvest, and storage [40].

Table 1: Summary of fungi isolated from stored pigeon pea (C*ajanus cajan)* using the direct plating method

Weeks of Storage	Fungal Species
Freshly prepared samples	A, C
4	A, B, C, F, H
8	A, B, C, D, F, H, I
12	A, B, C, D, F, G, H, I
16	A, B, C, D, F, G, H, I, J
20	A, B, C, D, F, G, H, I, J

Legend: A – *Aspergillus niger*, B – *Aspergillus fumigatus*, C – *Aspergillus flavus*, D – *Rhizopus stolonifer*, E – *Alternaria alternata*, F – *Fusarium oxysporum*, G – *Fusarium solani*, H – *Aspergillus oryzae*, I – *Fusarium moniliforme*, J – *Penicillium notatum*

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Table 2: Summary of fungi isolated from stored pigeon pea (C*ajanus cajan)* using the washing method

Weeks of Storage	Fungal Species
Freshly prepared samples	A, B
4	A, D, E, G, H
8	A, D, E, G, H, I
12	A, B, C, D, E, G, H, I
16	A, B, C, D, E, G, H, I, J
20	A, B, C, D, E, F, G, H, I, J

Legend: A – *Aspergillus niger*, B – *Aspergillus fumigatus*, C – *Aspergillus flavus*, D – *Rhizopus stolonifer*, E – *Alternaria alternata*, F – *Fusarium oxysporum*, G – *Fusarium solani*, H – *Aspergillus oryzae*, I – *Fusarium moniliforme*, J – *Penicillium notatum*

Table 3: Summary of fungi isolated from stored pigeon pea (*Cajanus cajan)* using the dilution method

Weeks of Storage	Fungal Species
Freshly prepared samples	B, C
4	A, B, C, D, F, H
8	A, B, C, D, E, F, H, I
12	A, B, C, D, E, F, G, H, I
16	A, B, C, D, E, F, G, H, I, J
20	A, B, C, D, E, F, G, H, I, J

Legend: A – *Aspergillus niger*, B – *Aspergillus fumigatus*, C – *Aspergillus flavus*, D – *Rhizopus stolonifer*, E – *Alternaria alternata*, F – *Fusarium oxysporum*, G – *Fusarium solani*, H – *Aspergillus oryzae*, I – *Fusarium moniliforme*, J – *Penicillium notatum*

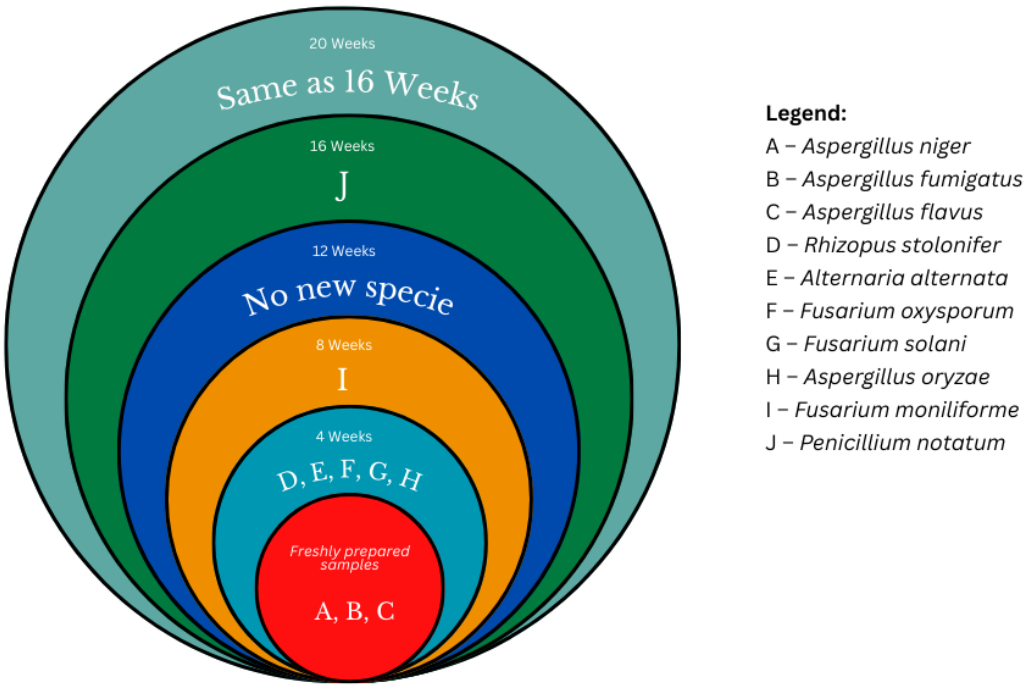

Figure 1: Fungi Isolated from Stored Pigeon Pea (*Cajanus cajan*) Across Different Storage Periods

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Table 4: Summary of results of proximate analysis of pigeon pea (*Cajanus cajan)* during 20 weeks of storage (g/100 g)

Weeks of Storage	**MC**	**Ash**	**Fat**	**CF**	**CP**	**CHO**
Freshly prepared	9.86 ± 0.02	2.60 ± 0.01	1.25 ± 0.01	7.75 ± 0.04	76.74 ± 0.05	0.22 ± 0.01
4	10.62 ± 0.03	2.89 ± 0.02	1.29 ± 0.02	6.50 ± 0.03	76.27 ± 0.04	0.63 ± 0.02
8	11.34 ± 0.03	2.97 ± 0.02	1.69 ± 0.02	5.96 ± 0.03	73.39 ± 0.03	1.51 ± 0.02
12	12.15 ± 0.03	3.14 ± 0.02	2.27 ± 0.02	5.28 ± 0.03	71.90 ± 0.03	4.86 ± 0.02
16	12.47 ± 0.03	3.14 ± 0.02	3.16 ± 0.02	4.82 ± 0.03	70.30 ± 0.03	8.00 ± 0.02
20	13.13 ± 0.02	3.15 ± 0.02	3.17 ± 0.02	4.61 ± 0.02	68.83 ± 0.02	10.56 ± 0.03

Legend: MC – Moisture content, Ash – Total ash, Fat – Crude Fat, CF – Crude fibre, CP – Crude protein, CHO – Carbohydrate.
Values are expressed as Mean ± Standard Deviation (SD) of triplicate determinations.

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Table 5: Summary of results of mineral analysis of pigeon pea (*Cajanus cajan)* during 20 weeks of storage (mg/100 g)

Weeks of Storage	**Na (mg/100g)**	**Ca**	**K**	**P**	**Mg**	**Mn**	**Fe**	**Zn**	**Pb**	**Cd**	**Ni**	**Cr**	**Cu**
Freshly prepared	3.49 ± 0.35	6.02 ± 1.09	21.26 ± 2.56	19.02 ± 2.85	11.75 ± 0.95	0.08 ± 0.01	0.28 ± 0.03	0.09 ± 0.01	0.00 ± 0.00	ND	ND	0.00 ± 0.00	0.07 ± 0.02
4 weeks	3.00 ± 0.35	5.52 ± 1.09	19.05 ± 2.56	11.85 ± 2.85	10.83 ± 0.95	0.08 ± 0.01	0.28 ± 0.03	0.07 ± 0.01	0.00 ± 0.00	ND	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02
8 weeks	2.66 ± 0.35	4.86 ± 1.09	16.85 ± 2.56	12.58 ± 2.85	10.77 ± 0.95	0.07 ± 0.01	0.23 ± 0.03	0.06 ± 0.01	ND	ND	ND	0.00 ± 0.00	0.05 ± 0.02
12 weeks	2.56 ± 0.35	4.03 ± 1.09	15.23 ± 2.56	11.85 ± 2.85	9.87 ± 0.95	0.06 ± 0.01	0.22 ± 0.03	0.06 ± 0.01	ND	ND	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
16 weeks	2.62 ± 0.35	3.45 ± 1.09	15.17 ± 2.56	12.07 ± 2.85	9.46 ± 0.95	0.05 ± 0.01	0.22 ± 0.03	0.06 ± 0.01	ND	ND	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
20 weeks	2.47 ± 0.35	3.43 ± 1.09	15.03 ± 2.56	12.03 ± 2.85	9.27 ± 0.95	0.05 ± 0.01	0.21 ± 0.03	0.05 ± 0.01	0.00 ± 0.00	ND	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02

Legend: Na – Sodium, Ca – Calcium, K – Potassium, P – Phosphorus, Mg – Magnesium, Mn – Manganese, Fe – Iron, Zn – Zinc, Cu – Copper, Pb – Lead, Cd – Cadmium, Ni – Nickel, Cr – Chromium, *ND – Not detected.

Values are expressed as Mean ± Standard Deviation (SD) of triplicate determinations.

The result of the proximate analysis of sundried pigeon pea (*Cajanus cajan*) seeds during 20 weeks of storage is shown in Table 4. There were changes in the nutritional value compared to the freshly pigeon pea seeds. This is due to fungal activity that caused changes during storage of the product [41]. After twenty weeks of storage, the moisture content, ash, Fat, and carbohydrate increased to 13.13, 3.15, 3.17, and 10.56 (g/100 g), respectively. On the other hand, the fibre and crude protein contents were decreased to 4.61 and 68.83 (g/100 g), respectively, during storage. These findings agreed with the report of Fagbohun and Ogundahunsi [23] who reported an increase in the moisture content (7.21-12.22), carbohydrate content (0.20-2.01), and a decrease in the fiber content (7.75 - 4.61) of stored sundried *Citrullus lanatus* Thunberg (Melon) Seeds. Fagbohun and Lawal [42] also reported an increase in the moisture content of sundried soybean (Glycine max.) from 6.80–8.34. High moisture levels enhance microbial growth, thereby shortening the shelf life of stored products [43]. An increase in moisture content and changes in other nutrient parameters during storage may be attributed to the activity of storage-associated mycoflora, which metabolize seed nutrients over time [41]. Fungal contamination and seed deterioration have been documented in legumes and other crops during prolonged storage, leading to associated losses in nutrient quality and the potential for mycotoxin production [42]. Changes in proximate composition, for instance, fluctuations in carbohydrate, Fat, fibre, and mineral content, have been observed in stored grains and pulses, highlighting that storage fungi can significantly alter seed chemistry [43]. For example, a recent study on seed-borne mycoflora of legumes under different storage conditions reported significant reductions in protein and mineral levels over time, correlated with increased fungal colonization [44]. Mineral analyses of stored pulses have shown that essential minerals such as K, Mg, Ca, Na, and trace elements decline as storage duration increases, likely due to fungal metabolism and associated biochemical transformations [45]. Comparable findings have been reported across different legumes and storage contexts, underscoring the need for rigorous storage hygiene and regular seed health monitoring to preserve nutritional quality [46].

CONCLUSION

Pigeon pea (*Cajanus cajan*) seeds are of great economic and nutritional importance. To preserve their quality and safety, they must be stored under controlled environmental conditions to prevent fungal contamination and nutrient deterioration. This study revealed that sun-dried pigeon pea seeds were contaminated with various fungal species during the 20-week storage period, leading to statistically significant ($p < 0.05$) reductions in key nutritional parameters, including crude protein, fiber, and mineral contents, while moisture, Fat, and carbohydrate levels increased. The isolated fungi, predominantly species of *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, and *Alternaria*, utilized the stored seeds as substrates, accelerating spoilage and nutrient degradation. These seed-borne fungi are known producers of spores and mycotoxins, which pose serious health risks to humans and animals when consumed through contaminated grains. Therefore, maintaining optimal storage conditions (low humidity, moderate temperature, and adequate ventilation) and implementing effective post-harvest handling practices are critical to minimizing fungal proliferation, preserving nutritional quality, and preventing economic losses associated with pigeon pea storage.

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Antidiabetic and Nephroprotective Effects of Aqueous Leaf Extracts of *Moringa oleifera* in Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus (DM) is a global burden, associated with life-threatening complications which include renal failure, cardiac attack and stroke. This study was carried out to investigate the antidiabetic and nephroprotective effects of aqueous leaf extracts of *Moringa oleifera* in rats with alloxan-induced diabetes. For this purpose, twenty-five adult Wistar rats with body weight of 150-250g were randomly assigned to groups of five rats each (n=5). Group A served as normal control; group B served as diabetes control; group C served as positive control and group D and E were diabetic groups administered with the aqueous leaf extracts. Blood samples were collected to determine the fasting blood glucose level, creatinine and urea. The results from this study showed that the aqueous leaf extract of *Moringa oleifera* demonstrated significant antidiabetic activity by reducing blood glucose levels by 34.7% and 36% when lower and higher doses of extracts of *M. oleifera* were administered to rats respectively and also showed an improvement in glucose tolerance in the diabetic rats. Additionally, the extract demonstrated nephroprotective effects by reducing markers of kidney damage and improving kidney functions in the diabetic rats. This was evident in the reduction of urea and Creatinine levels when compared to positive and negative control groups. The findings suggest that *M. oleifera* leaf extracts may have potential therapeutic benefits for managing diabetes and protecting against kidney damage. Further research on other parts of the plant is recommended to elucidate its mechanism of action and active compounds.

Keywords: Alloxan; Hyperglycemia; Hypoglycaemic; *Moringa oleifera;* Wistar rat

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INTRODUCTION

Diabetes mellitus [DM] is one of the top ten causes of morbidity and mortality around the world [1]. It is a metabolic disorder of the endocrine system which is characterized by hyperglycemia, dysregulations in insulin levels, with alterations in protein, carbohydrate and lipid metabolism and in some cases, it occurs concurrently in individuals thereby leading to obesity and overweight [2,3].

The four major classifications of diabetes are Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes and secondary diabetes. However, the vast majority of people with diabetes are classified into one of two broad classes: T1DM, which is caused by an absolute or near absolute deficiency of insulin, or T2DM which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. Others are women who develop diabetes during their pregnancy and are classified as having gestational diabetes. Lastly, there are uncommon and diverse types of diabetes, which are caused by drugs, infections, pancreatic destruction, endocrinopathies and genetic defects. These unrelated forms of diabetes are included in the "Other Specific Types" that are secondary type of diabetes and are classified separately [4,5].

Recently, approximately 150 million people have diabetes worldwide, a number expected to reach 300 million by 2025 [6]. Currently, the available remedy or management of diabetes involves treatment with insulin and other oral anti-diabetic agents such as metformin, sulfonylurea and biguanid. Many of these oral anti-diabetic agents have a number of serious with adverse effect, thus management of diabetes without side effect is still a challenge [7]. The rapid increase in cases of diabetes mellitus, most especially T2DM, raises concerns, and also highlights an urgent need to investigate effective therapies to curb this disease [8].

Herbs have become reliable substitutes and have played a significant role in the management of various disorders. One of these herbs is *Moringa oleifera. Moringa oleifera,* popularly called "the miracle tree", is a monogeneric plant of the family *Moringaceae* [9]. It is a medicinal plant that has gained a lot of interest for its diverse phytochemical properties. A review of available literature indicates the biological capabilities of this plant expand to protecting against complications linked with cancer, heart disease, diabetes mellitus and fatty liver [10,11].

Different parts of moringa plant contain important minerals such as K, Ca, P, Fe, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics such as zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol and high concentrations of natural dietary antioxidants: Vitamins A, C and E [12]. The leaves are known to be used mostly for medicinal purpose, and they are a great source of antioxidant flavonoids and prominent anti-inflammatory [13].

Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes mellitus due to their effectiveness, less side effects and relatively low cost. However, most of the plants prescribed for diabetes mellitus are not edible and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease. Therefore, this study aimed at investigating the antidiabetic and nephroprotective effect of aqueous leaf extracts of *Moringa oleifera* in alloxan-induced diabetic rats.

METHODOLOGY

Sample Collection and Preparation

The plant materials *(*leaves of *Moringa oleifera)* were sourced from Nasarawa town in Nasarawa Local Government Area of Nasarawa State, Nigeria. The identification and authentication were done by a taxonomist in the department of Agricultural Technology, Federal Polytechnic, Nassarawa and the voucher number was assigned and deposited in the same Department. The preparation of the leaves sample was carried out using the method of Josiah *et al.* [14]. The sample was washed under running tap water and then air dried on top of the laboratory bench and thereafter crushed with a mortar and pestle into fine powder. The powdered leaves (500 g) were extracted by cold maceration in distilled water for 48 hours at room temperature with manual intermittent agitations after every 3 hours. Thereafter, the extract was filtered using Whatmann No. 1 filter paper and concentrated at 40˚C in a hot air oven. The resulted extract was stored in a capped bottle in refrigerator at 4˚C until required.

The Experimental Animals and Design

The method of Josiah *et al*. [15] was used to calculate the sample size. A total of twenty-five (25) wistar strain of albino rat (WSAR) of both sexes, weighing between 150-250 g were obtained from the animal house of the Department of Pharmaceutical Science, Ahmadu Bello University (ABU) Zaria, Nigeria. The rats were kept and maintained in well-ventilated cages under standard laboratory conditions and were maintained at ambient temperature and relative humidity which was between 22°C, 12 hours light and 12-hour dark and 30-35% humidity respectively [16]. They were maintained on grower's mash (Vital feeds Nigeria Ltd) and

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provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the experiment [14]. The animal ethical committee in the Department of Laboratory Technology, Federal Polytechnic, Nasarawa approved the animal studies.

After the acclimatization, the 25 WSAR rats were randomly divided into five groups of five rats each:

Group A: Normal rats administered with only distilled water (positive control).

Group B: Diabetic-induced rats administered without treatment (negative control).

Group C: Diabetic-induced rats administered with a standard drug (metformin).

Group D: Diabetic-induced rats administered with 200 mg/kg aqueous extract of *Moringa oleifera* (Low Dose)

Group E: Diabetic-induced rats administered with 400 mg/kg of aqueous extracts of *Moringa oleifera* (High Dose)

After 28 days of administration, the animals were humanely sacrificed and blood was collected.

Induction of Diabetes
After the rats were fasted for 18 hours, they were induced by single intraperitoneal injection of freshly prepared Alloxan (120 mg/kg) in 0.1 M citrate buffer (pH 4.5) for type 2 diabetes [17]. Diabetes were confirmed in these rats after a period of 7 days. The control animals were administered citrate buffer (pH 4.5). To overcome the initial hyperglycaemic shock, which may occur as a result of the Alloxan administration, diabetic rats were given 5% glucose solution ad libitum for 24 hours. The method of Okoli *et al.* [17] with modification was used to collect the blood from the tail and the glucose level of each rat was determined. Rats with a fasting blood glucose range of 15–20 mmol/L were considered diabetic and included in the study [17] The fasting blood glucose level was determined using glucometer (Acc-check Advantage. Boche Diagnostic GmbH, Germany) after fasting the rats for 12 hours [18].

Collection for Biochemical Analysis of Hepatic and Renal Functions
Blood samples were collected during the experiment via retro-orbital bleeding under light ether anaesthesia. Blood samples collected were left to clot at room temperature, and then centrifuged at 3000 rpm for 15 min. Sera were then separated and stored at -20 °C as aliquots for further biochemical analysis [19].

Sera were used to evaluate serum levels of hepatic and renal function biomarkers. Serum creatinine and urea were determined according to Larsen *et al.* respectively [20, 21]. Rats were administered with the extracts at various concentrations daily for four weeks experimental period, while blood glucose level was determined weekly for the period of four weeks. The rats were fasted overnight and anesthetized with chloroform and were sacrificed 24 hours after the last treatment. The serum collected was used to determine blood urea nitrogen (BUN), and creatinine. Creatinine present in the serum was determined using the Randoxlaboratories^{TM} assay kit as described by Myes *et al.* [22]. Serum urea concentration was measured using spectrophotometer (UV-V is spectrophotometer - Gold Spectrum lab 54 produced from Angstron Advanced Inc, Buston USA) according to Berthelot's reaction, using (Randox assay kit) according to Fawcett and Scout [23].

Data Analysis
The results were presented as mean±SEM of 5 rats in each group. Analysis of variance (ANOVA) was used to test for significance differences between the treated and untreated groups. Values of $P< 0.05$ was considered significance and Multiple comparism test (Tukey's Honestly Significant Difference (HSD) test) was used [24].

RESULTS
The results indicate that aqueous leaf extracts of *Moringa oleifera* exhibit significant hypoglycemic activity in alloxan-induced diabetic Wistar rats. This is evidenced by the reduction in fasting blood glucose levels, as shown in Table 1. A significant difference was observed between pre- and post-treatment glucose levels in all groups except Group A. The percentage reduction of blood glucose level of the treatment of alloxan-induced diabetic rats with metformin and different concentration of aqueous extracts of *M. oleifera* are shown in Table 2. The groups (D and E) treated with extract of *M. oleifera* had reduction of blood glucose level at 34. 7% and 36% respectively while the highest reduction of blood glucose level at 44.1% was found in group C treated with metformin which is the standard drug. On the other hand, group A which was positive control, showed increase in blood glucose level while group B which was negative control showed reduction in blood glucose level at 2.9% and 18.6% respectively.

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Table 1: Effect of aqueous leaf extracts of *Moringa oleifera* on fasting blood sugar level in albino wistar rats

Treatment	Initial (mg/dl)	After induction (mg/dl)	After treatment (mg/dl)
Group A	83.7±15.65^{a}	86.3±16.34^{ab}	88.8±16.67^{ab}
Group B	86.9±16.37^{a}	157.3±26.53^{c}	128±23.60^{b}
Group C	90.5±18.65^{a}	259.3±37.98^{c}	145±25.85^{b}
Group D	85.4±16.26^{a}	194.9±30.53^{c}	127.2±23.46^{b}
Group E	81.2±14.92^{a}	184.3±28.50^{c}	118±21.70^{b}

Result= mean value ± SD of duplicate determinations. Different superscript in a row means that the difference is significant at probability value p = 0.05 (95% confidence interval), otherwise, there is no significant difference.

Group A= Normal control (Positive)
Group B = Negative Control
Group C = Metformin
Group D = DE + 200mg/kg
Group E = DE +400mg/kg

Table 2: Percentage reduction of blood glucose level after treatment of experimental animals with aqueous leaf extracts of *M. oleifera* and metformin

Treatment	After induction (%)	After treatment (%)
Group A	100	-2.9
Group B	100	18.6
Group C	100	44.1
Group D	100	34.7
Group E	100	36

Note: Group A which is positive control shown 2.9% increase in blood glucose level

Table 3 shows the effect of aqueous leaf extracts of *M. oleifere* on urea and creatinine level in WSAR. The result indicates reduction in the value of urea and creatinine of WSAR treated with *M. oleifera* when compared to normal and metformin control and with significant difference. The values of creatinine also showed reduction in the WSAR rats treated with the leaf extract of M. *oleifera* when compared to the normal control with significant difference. However, the value of metformin control was lower than the values of creatinine treated with aqueous leaf extracts with significant difference.

Table 3: Effect of aqueous leaf extracts of *Moringa oleifera* on urea and creatinine levels in Wistar rats

	NC	DC	MC	LD	HD
UREA	24.0 ± 3.51^{a}	14.0 ± 1.41^{b}	29.2 ± 3.44^{a}	11.1 ± 1.72^{b}	12.7 ± 2.05^{b}
CREATININE	0.70 ± 0.070^{ad}	0.83 ± 0.124^{a}	0.10 ± 0.078^{c}	0.45 ± 0.111^{bcd}	0.58 ± 0.181^{ab}

Key: NC = Normal control; DC = Diabetic control; MC = Metformin control; LD = Low dose; HD = High dose; Different superscript in a row means that the difference is significant at p<0.05

DISCUSSION

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both [25]. The results obtained suggest that aqueous leaf extracts of *Moringa oleifera* exhibited significant hypoglycemic activities in alloxan induced diabetic Wistar rats. This was evidenced by reduction of fasting blood glucose level observed in Table 1. The significant increase in serum glucose levels in the diabetic control group (86.9 ± 16.37a to 157.3 ± 26.53c) compared to the normal control group (83.7 ± 15.65a to 86.3 ± 16.34ab) may be attributed to alloxan administration, which damages pancreatic β-cells. The administration of alloxan monohydrate which are known to induces diabetes by damaging insulin secreting cells of the pancreas leads to hyperglycemia [26, 27]. There is selective necrosis of the β-cell of the islet of langerhans on the pancreas so that the production of insulin is totally or partially inhibited, which is dependent on the concentration of the alloxan [28].

There was significant ($p<0.05$) decrease in the level of fasting blood glucose in diabetic rats with 34.7% and 36% reduction of rats treated with 200 mg/kg and 400 mg/kg of aqueous leaf extract of *M. oleifera* respectively when compared to group C treated with conventional drug (metformin) with 44.1% reduction in blood glucose level. The Treatment with metformin (which is the drug of reference) recorded a non-significant but slightly higher hypoglycemic effect (44.1%) when compared with the aqueous leaf extracts within the same period of time.

The findings in this study agreed with the findings of Ezeigbo *et al.* [16] and Sai *et al.* [29] who revealed clearly that the aqueous extract of *M. oleifera* leaf possesses antihyperglycemic and antihyperlipedemic effect in both insulin deficient and insulin resistant in rat models. The hypoglycaemic influence of the *M. oleifera* observed in the present study is also in conformity with Hegde *et al.* who observed 36% reduction in blood glucose levels in alloxan induced diabetic rats maintained on the *M. oleifera* [30]. Varga-Sanchez *et al.* also had a similar result in *reduction* of blood glucose level after 6 weeks experimental duration by feeding rats with the *M. oleifera* leaves extract [31]. The hypoglycemic activity of the extract could be due to pancreatic cell regeneration that were partially destroyed by alloxan, which may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose [32, 33].

Diabetic hyperglycemia is known to induce elevated blood urea and serum creatinine levels, which serve as significant markers of renal dysfunction [34]. The balance impairment of nitrogen coupled with reduction in protein synthesis can leads to increase in concentration of urea in the blood and the increased in plasma creatinine level and blood urea are indication of the development of diabetic nephropathy in rats [35].

In the present study (Table 3), there was a significant reduction in the blood urea and serum creatinine levels from the group treated with low dose (11.1 ± 1.72 and 0.45 ± 0.111) and high dose (12.7 ± 2.05 and 0.58 ± 0.181*)* of aqueous leaf extracts *of M. oleifera* respectively, when compared with normal control group (24.0 ± 3.51 and 0.70 ± 0.070). This study revealed that the administration of alloxan to rats prevented the development of diabetic nephropathy by lowering blood urea and serum creatinine. This could be explained that there was increased clearance of blood urea and creatinine in the kidney due to antioxidant or anti-inflammatory properties of *M. oleifera* which might contribute to renal protection or that there was decreased protein degradation. This result agrees with the findings of Gross *et al.* [36].

It was observed in this study that there was increase in urea concentration (29.2 ± 3.44) and decrease in creatinine concentration (0.10 ± 0.078) of the metformin control group when compared with the diabetic control group for urea and creatinine (24.0 ± 3.51 and 0.70 ± 0.070). This was also observed by Hasan *et al*. [37]. This report suggested that *Crateva adansonii* prevented the progression of diabetic nephropathy and protected the kidney from further damage. The end-stage of diabetic renal disease is usually characterized by changes in both proteinuria and subsequent gradual decline in glomerular filtration rate (often progressing over 10–20 years). Therefore, the development of lesions in the glomerular capillaries of the kidneys allows protein to escape because of changes in the basement.

CONCLUSION

The aqueous leaf extracts of *Moringa oleifera* exhibited promising hypoglycemic and nephroprotective effect in alloxan induced diabetic wistar rats. Hence the leaf can be helpful in the management of diabetes mellitus and its other associated complication. It is recommended that further investigation using other part of the plant may be more useful to explicate the mechanism of action and other active principles to project this plant as a therapeutic target in diabetic research.

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ETHICAL APPROVAL
The animal ethical committee in the Department of Laboratory Technology, Nasarawa Federal Polytechnic approved the animal studies with Protocol number: H20371. The principles of the laboratory animal care were followed.

CONFLICT OF INTEREST
The authors declare no conflicts of interest in this research.

AUTHOR'S CONTRIBUTION
JJG and JZ designed the experiment, oversaw data collection and execution, and contributed to manuscript preparation; TMN participated in experimentation, collation of data and manuscript preparation; AJY participated in design of experiment and manuscript preparation; ESS and MJT participated in sample collection, experimentation and data collation

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LIST OF ABBREVIATIONS
ANOVA: Analysis of Variance; BUN: Blood Urea Nitrogen; DC: Diabetic Control; DM: Diabetes Mellitus; HD: High Dose; HSD: Honestly Significant Difference; IDF: International Diabetes Federation; LD: Low Dose; MC: Metformin Control; NC: Normal Control; SEM: Standard Error of Mean; STZ: Streptozotocin; T1DM: Type 1 Diabetes Mellitus; T2DM: Type 2 Diabetes Mellitus; WHO: World Health Organization; WSAR: Wistar Strain of Albino Rats.

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