Isolation and Characterization of Multidrug Resistant Bacteria from Pig Farms in Ibadan: One Health Approach

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In Partial Fulfilment of the Requirements for the Award of Masters of Science Degree (MSc) in Medical Microbiology

2025

Chapter One

Introduction

1.1 Background to the Study

Antibiotic resistance occurs when bacteria evolve mechanisms to withstand the drugs that could treat infections caused by these bacteria[2]. This renders treatments for ailments ineffective, leading to persistent infections, increased transmission of resistant bacteria, and higher medical costs and mortality rates[3]. When bacteria develop defenses against the medications used to treat their infections, antibiotic resistance develops [2]. This makes illness treatments inefficient, which raises medical expenses and mortality rates, increases the spread of resistant germs, and causes chronic infections [3]. There are multiple factors that contribute to the development of antibiotic resistance. This threat is exacerbated by the overuse and abuse of antibiotics in agriculture and human medicine. In human medicine, incomplete antibiotic courses, inadequate dosage, and poor prescribing all contribute to the survival of resistance strains[1]. Similar to this, antibiotics are frequently used in agriculture for prophylactic and growth-promoting goals in addition to therapeutic ones, which exacerbates the issue [5].

Globally, antibiotic resistance is recognized as a critical issue especially by the World Health Organization (WHO), which has declared it one of the top ten global public health threats. Efforts to combat antibiotic resistance include the development of new antibiotics, antimicrobial stewardship programs to promote appropriate use, and global surveillance systems to monitor resistance patterns [6]. The study of multidrug-resistant (MDR) bacteria is crucial for several reasons. MDR bacteria are defined as strains that exhibit resistance to multiple antibiotics, making infections caused by these organisms particularly difficult to treat[6]. Understanding the

mechanisms and drivers of multidrug resistance is essential for developing effective strategies to mitigate its spread and impact [7].

Bacteria acquire resistance through various mechanisms, some of which include mutation and horizontal gene transfer [6]. Mutation leads to genetic changes that can confer resistance on bacterial isolates, while horizontal gene transfer allows for the exchange of resistance genes between bacteria, often though plasmids, transposons, integrons or other mobile genetic elements. This genetic exchange can occur across different species and environmental settings, amplifying the spread of resistance[7].

The implications of antibiotic resistance are profound. It threatens the efficacy of routine medical procedures such as surgeries, cancer treatments, and the care of chronic diseases, as these procedures rely heavily on effective antibiotics for infection prevention and treatment [8]. Moreover, resistant infections can cause prolonged hospital stays, requiring more intensive care and alternative, often more toxic and expensive, treatment options [9].

MDR bacteria pose a significant threat to public health. Infections caused by these organisms are associated with higher morbidity and mortality rates due to limited treatment options. Studying MDR bacteria helps identify the extent of the problem and informs public health policies to address it effectively[12]. In the agricultural sector, especially in livestock farming, the use of antibiotics is widespread and it is a cause for concern. The unregulated use of antibiotics contributes to the development and dissemination of MDR bacteria, which can be transferred to humans through direct contact, environmental pathways, or the food chain. Investigating MDR bacteria in agricultural settings, such as pig farms, is vital to understand and curb this transmission route [13,14].

The increasing global demand for animal protein has led to a significant rise in pig farming, an essential agricultural activity providing substantial economic benefits and contributing to food security [9]. Pigs, being highly efficient converters of feed to meat, play a crucial role in meeting the protein needs of a growing population. Pork meat, derived from pigs, is a staple in many diets worldwide due to its affordability, palatability, and nutritional value [9]. In regions like Ibadan, Nigeria, piggery farms are pivotal in sustaining local economies and supplying fresh pork to the market.

However, intensive pig farming practices often involve the extensive use of antibiotics for disease prevention and growth promotion [10]. This indiscriminate antibiotic use has led to the emergence and proliferation of multidrug-resistant (MDR) bacteria within pig populations. These bacteria pose significant risks not only to animal health but also to public health, emphasizing the interconnectedness of human, animal, and environmental health a concept encapsulated in the One Health approach [11]. This study aims to isolate and characterize multidrug-resistant bacteria from pig farms in Ibadan, providing insights into the prevalence of resistance and informing strategies to mitigate the spread of MDR pathogens within the pig farming industry and beyond.

1.2 Statement of the Problem

Pig farms are critical in the food production chain, yet they are also potential reservoirs for multidrug resistant (MDR) bacteria [1]. The intensive use of antibiotics in livestock farming to promote growth and prevent disease has led to the emergence and proliferation of MDR bacteria. In pig farms, farming practices which include the use of antibiotics can create environments where bacteria are frequently exposed to antimicrobial agents, selecting for strains that can resist multiple drugs. These MDR bacteria can thrive in the pigs themselves, as well as in the farm environment, including soil, water, and air. MDR bacteria can spread easily within a pig farm

through direct contact between animals, shared equipment, and contaminated feed or water. Workers in pig farms are at high risk of acquiring MDR bacteria through direct contact with animals or contaminated environments. This transmission can also extend to farm visitors and the surrounding community[15].

Waste products from pig farms, such as their dung and urine which can be used as manure, can introduce MDR bacteria into the surrounding environment. These bacteria can contaminate water sources, soil, and crops, further spreading resistance genes through ecological systems. MDR bacteria make it challenging to treat common infections in pigs, leading to higher morbidity and mortality rates, which can adversely affect farm productivity and economic viability [15].

The study of MDR bacteria in pig farms is highly relevant to public health due to the potential for zoonotic transmission where diseases can be transferred from animals to humans. The presence of MDR bacteria in livestock farms poses a significant threat to both animal and human health. It challenges the effectiveness of antibiotics, which are critical for treating bacterial infections. The spread of these resistant bacteria can lead to infections that are difficult, if not impossible, to treat with existing medications [14]. The study of MDR is pig farms is important as it will provide data on the types of bacteria showing resistance in pig farms and the possibility and manner of transmission of these bacteria to humans either in pork meat, contact with farm hands or even through run off into rivers and water bodies around the pig farm [19].

The One Health approach is particularly important in this context. One Health is a collaborative, multi-sectorial, and transdisciplinary approach that recognizes the interconnection between people, animals, plants, and their shared environment [15]. The key principles of One Health emphasizes that the health of animals, humans, and ecosystems are interconnected [16]. The spread of MDR bacteria in pig farms impacts the entire ecosystem, thereby making it necessary that a

comprehensive approach to understanding and mitigating risks is adopted. Addressing MDR bacteria requires collaboration across various disciplines and sectors, including veterinary medicine, human medicine, environmental science, and public health policy[18].

1.3 Justification of the Study

The emergence and rapid dissemination of multidrug-resistant (MDR) bacteria represent a critical global health concern that transcends human and veterinary medicine. Pig farms, which play an integral role in the livestock and food production chain, have been increasingly recognized as potential hotspots for the development and spread of antibiotic-resistant pathogens. In Nigeria, the extensive and often unregulated use of antibiotics in animal husbandry particularly for prophylaxis, therapy, and growth promotion has contributed significantly to the selection pressure driving antimicrobial resistance (AMR) among bacterial populations.

Despite growing awareness of AMR as a global threat, limited empirical data exist on the distribution, resistance profiles, and transmission dynamics of MDR bacteria within pig farming systems in Nigeria, especially in the Ibadan metropolis. This knowledge gap hinders the development of evidence-based interventions and surveillance strategies. Therefore, this study is justified by the urgent need to generate local data on the prevalence, phenotypic, and genotypic characteristics of MDR bacteria circulating within pig farm environments.

Focusing on *Escherichia coli* and *Staphylococcus aureus* two clinically and epidemiologically significant pathogens this research seeks to elucidate the resistance mechanisms and potential cross-transmission routes between animals, humans, and their shared environment. By adopting the One Health approach, the study integrates human, animal, and environmental perspectives to provide a holistic understanding of antimicrobial resistance dynamics in agricultural ecosystems.

Furthermore, the findings of this study will provide essential baseline data that can inform antimicrobial stewardship programs, guide veterinary public health policies, and enhance biosecurity and hygiene practices on farms. The research outcomes will not only support national and global efforts to mitigate the burden of antimicrobial resistance but will also contribute to sustainable livestock production, food safety, and environmental health.

In essence, this study is justified by its potential to bridge critical data gaps, strengthen intersectoral collaboration, and promote evidence-based interventions aimed at curbing the spread of multidrug-resistant pathogens within the pig farming industry and the broader ecosystem.

1.4 Aim and Objectives of the Study

The major aim of this study is to isolate and characterize multidrug resistant (MDR) bacteria isolated from samples collected on pig farms in Ibadan, utilizing the One Health approach to understand the prevalence, and resistance profiles in these bacterial isolates.

The specific objectives are to:

1. Isolate MDR *Escherichia coli* and *Staphylococcus aureus* from various sources within pig farms in Ibadan, including pig feces, feed, water sources, and environmental samples.
2. Characterize the isolated MDR bacteria using phenotypic and genotypic methods.
3. Determine the resistance profiles of the isolated MDR bacteria against a panel of clinically relevant antibiotics, including those commonly used in human healthcare.
4. To investigate whether MDR bacteria isolated from pig farms are also resistant to commonly used farm disinfectants

5. Provide evidence-based recommendations for mitigation strategies aimed at reducing the prevalence and spread of MDR bacteria in pig farms, including improved antibiotic stewardship, biosecurity measures, and environmental management practices.

1.5 Research Questions

1. What is the prevalence of multidrug resistant (MDR) bacteria in diverse environmental samples (soil and water), feces, collected from pig farms in Ibadan, in line with the One Health framework?
2. Which bacterial species, present in different environmental niches within pig farms, harbor multidrug resistance genes, and how does this distribution correlate with human and animal interaction points?
3. How do the antimicrobial resistance profiles of MDR bacteria isolated from feces, soil, water, and other environmental samples compare against used for both veterinary and human medicine?
4. What are the primary pathways of MDR bacteria transmission within pig farms, considering interactions between animals, humans, and the farm environment, and how does this knowledge inform cross-disciplinary prevention strategies?
5. What are the public health implications of MDR bacteria isolated from various environmental sources within pig farms, and how does this impact antibiotic treatment efficacy across both veterinary and human healthcare domains, aligning with the One Health perspective?

1.6 Significance of the Study

This study holds significance at various levels. The emergence and spread of multidrug resistant (MDR) bacteria pose a serious threat to public health globally. By focusing on pig farms in Ibadan, where antimicrobial resistance is a growing concern, this study will provide crucial insights into the prevalence, distribution, and characteristics of MDR bacteria in the local environment. Understanding the dynamics of MDR bacteria transmission within pig farms is essential for developing targeted interventions to mitigate public health risks associated with zoonotic transmission and antibiotic resistance[9].

This study in alignment with the One Health approach, recognizing the interconnectedness of human, animal, and environmental health. By investigating MDR bacteria in diverse environmental samples from pig farms, the study addresses the interactions between animals, humans, and their shared environment. This holistic perspective is important for developing strategies to combat antimicrobial resistance and safeguard public health.

The findings of this study will bridge the gap between veterinary and human medicine by comparing antimicrobial resistance profiles of MDR bacteria isolated from pig farms against antibiotics commonly used in both sectors[19]. This comparative analysis will enhance our understanding of cross-species transmission dynamics and inform evidence-based antimicrobial stewardship practices in both veterinary and human healthcare settings. The research outcomes will provide valuable data to policymakers, veterinarians, healthcare professionals, and farm managers for implementing effective strategies to combat antimicrobial resistance in pig farming[10]. Recommendations derived from this study can inform the development of policies and guidelines aimed at promoting prudent antibiotic use, improving biosecurity measures, and enhancing surveillance systems to monitor and control MDR bacteria in agricultural settings [20].

Antimicrobial resistance is a global health security threat that transcends national borders [15]. By generating local data on MDR bacteria prevalence and transmission pathways, this study contributes to the global effort to combat antimicrobial resistance. The insights gained from this research can inform international initiatives and collaborations aimed at addressing antimicrobial resistance and preserving the effectiveness of antibiotics for future generations. By generating data on MDR bacteria prevalence, transmission, and impacts, it contributes to the global effort to combat antimicrobial resistance, promoting scientific advancement, agricultural productivity, and population well-being

The significance of this study is also in its contribution to advancing knowledge on antimicrobial resistance in pig farming environments, its implications for public health within the One Health framework, and its potential to inform evidence-based interventions to mitigate the threat of multidrug resistant bacteria and promote sustainable agricultural and healthcare practices.

1.7 Scope of the Study

The study focuses on pig farms located within the vicinity of Ibadan, Nigeria. Sampling and data collection will be conducted exclusively within this geographical area to provide insights into the local prevalence and dynamics of multidrug resistant (MDR) bacteria in pig farming environments. The scope of the study encompasses various environmental samples collected from pig farms, including pig feces, soil, water sources, and potentially other relevant matrices. By examining MDR bacteria in diverse environmental niches, the study aims to comprehensively assess the presence and distribution of antimicrobial resistance within the farm ecosystem.

Antimicrobial resistance profiles of MDR bacteria isolated from pig farms will be compared against antibiotics commonly used in both veterinary and human medicine. This comparative

analysis enables insights into cross-species transmission dynamics and informs antimicrobial stewardship practices across veterinary and human healthcare sectors.

1.8 Limitations of the Study

The limitations of the study include the fact that the study's findings may be influenced by sampling bias, as samples are collected from specific pig farms in Ibadan. While efforts will be made to ensure representative sampling, variations in farm management practices and environmental conditions may impact the generalizability of results to other pig farming regions.

During sample collection and processing, there is a risk of cross-contamination, potentially leading to erroneous results. Strict adherence to standardized protocols and quality control measures will be implemented to minimize the risk of contamination and ensure the reliability of study outcomes. The scope of the study may be constrained by limited resources, including funding, personnel, and time. Resource limitations may impact the scale of sampling, depth of analysis, and breadth of data interpretation, potentially limiting the study's ability to fully capture the complexity of antimicrobial resistance dynamics in pig farming environments.

1.9 Operational Definition of Terms

Antibiotic Resistance: The ability of bacteria to survive and grow in the presence of drugs that were previously effective against them.

Multidrug-resistant (MDR) Bacteria: Bacteria that exhibit resistance to multiple antibiotics, making infections caused by these organisms difficult to treat.

Horizontal Gene Transfer: The movement of genetic material between bacteria through mechanisms such as plasmids, transposons, and integrons, allowing for the spread of resistance genes.

One HealthApproach: A collaborative, multi-sectoral, and transdisciplinary approach that recognizes the interconnectedness of the health of people, animals, plants, and their shared environment.

Antimicrobial Stewardship: Programs and strategies aimed at promoting the appropriate use of antibiotics to reduce the development of resistance.

Zoonotic Transmission: The transfer of diseases from animals to humans.

Biosecurity Measures: Practices and procedures implemented to protect against the spread of infectious diseases.

Resistance Profiles: The patterns of resistance exhibited by bacteria against a range of antibiotics.

Integrated management practices: Coordinated strategies that involve multiple sectors to manage health risks and promote sustainability.

Cross-species transmission: The spread of diseases or resistance genes between different species, including humans and animals.

Endnotes

1. O.S. Akinsuyi, O.Q. Orababa, O.M. Juwon, I.O. Oladunjoye, E.T. Akande, M.M. Ekpueke & A. Emmanuel, *One Health approach, a solution to reducing the menace of multidrug-resistant bacteria and zoonoses from domesticated animals in Nigeria–A review*, **Global Biosecurity**, 3, 2021, pg. 6.

2. A.P Akinduti, O. Ayodele, B.O. Motayo, Y.D. Obafemi, P.O. Isibor & O.W. Aboderin, *Cluster analysis and geospatial mapping of antibiotic resistant Escherichia coli O157 in southwest Nigerian communities*. **One Health,** *15*, 2022, pg. 100447.

3. O.S. Fasiku, O.O. Omotosho, S.O. Ajulo, E.E. Adesanwo, O.D. Oni, A.A. Awoyele, O.A. Okunlade & V.O. Adetunji, *Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus in Pigs in Ibadan, Nigeria.* **In Medical Sciences Forum** (Vol. 24, No. 1), 2023, pg. 8.

4. O.I. Falodun, N.G. Buekor & A. Victor *Multidrug-resistant Enterococcus and coagulase-negative Staphylococcus isolates from poultry farms and nearby wells in Ibadan, Nigeria and their resistant genes*. **One Health Bulletin**, *4*(3), 2024, pg. 117-123.

5. K.O.E. Ada, E.C. Clare, O.E.S. Chidinma , U.O. Goodluck & A. Ei, *Isolation of multidrug resistant and extended spectrum β-Lactamase producing bacteria from faecal samples of piggery farms in Anambra State, Nigeria*. **Am J Infectious Dis Microbiol,** *9*(4), 2021, pg.106-113.

6. O.S. Egbule, B.C. Iweriebor & E.I. Odum, *Beta-Lactamase-producing Escherichia coli isolates recovered from pig handlers in retail shops and Abattoirs in selected localities in Southern Nigeria: Implications for public health.* **Antibiotics,** *10*(1), 2020, pg. 9.

7. J. Muleme , C. Kankya, M. Munyeme, D. Musoke, J.C. Ssempebwa, J.B. Isunju, R. Wambi, B.E. Balugaba, T. Sekulima, R.K. Mugambe, & S. Cadmus, *Phenotypic Characterization and Antibiograms of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolated at the Human-Animal-Environment Interface Using a One Health Approach Among Households in Wakiso District, Uganda.* **Infection and Drug Resistance,** 2, 2023, pg. 2203-2216.

8. D.A. Gado, D.O. Ehizibolo, C.A. Meseko , N.E. Anderson, & P.W. Lurz, *Review of emerging and re-emerging zoonotic pathogens of dogs in Nigeria: Missing link in one health approach*. **Zoonotic Diseases**, *3*(2), 2023, pg. 134-161.

9. N.O. Oloso , S. Fagbo, M. Garbati, S.O. Olonitola, E. J. Awosanya, M.K. Aworh, H. Adamu, I.A. Odetokun, & F.O. Fasina, *Antimicrobial resistance in food animals and the environment in Nigeria: A review.* **International Journal of Environmental Research and Public Health,** *15*(6), 2018, pg. 1284.

10. O.I. Falodun, & A.A. Ameh. *Multidrug resistance and virulence genes in Campylobacter isolates from poultry waste in Ibadan, Nigeria.* **One Health Bulletin**, *4*(1), 2024, pg. 39-46.

11. I.H. Igbinosa, C.N. Amolo, A. Beshiru, O. Akinnibosun, A.G. Ogofure, M. El-Ashker, M. Gwida, A.I. Okoh & E.O. Igbinosa, *Identification and characterization of MDR virulent Salmonella spp isolated from smallholder poultry production environment in Edo and Delta States, Nigeria.* **PloS One,** *18*(2), 2023, pg. 281-329.

12. O.E. Ojo, A.M. Iledare, E.A. Amosun, & M.A. Dipeolu. *Antimicrobial use and detection of cefotaxime-resistant Enterobacteriaceae in the pig production chain, Ogun State, Nigeria.* **Revue d'élevage et de médecine vétérinaire des pays tropicaux,** *72*(4), 2019, pg. 147-154.

13. A.M. Ndahi, I.O. Fagbamila, I. Adebiyi, E. Ekeng, S. Adeleke, V. Adetunji, R.S. Hendriksen, C. Ryan, H. Patel, T. Chisnall, & D. Verner-Jeffreys, *Establishing a One Health AMR Community of Practice in Nigeria. One Health Cases*, 2023, 2, pg. 30.

14. A.O. Ajayi, F.C. Samuel-Osamoka, O.P. Adejoh, & O.A. Popoola, *Antibiotic resistance among bacteria isolated from pigs in selected farms in Ekiti State, Nigeria*, **Dutse Journal of Pure and Applied Sciences,** *9*(4b), 2023, pg. 337-346.

15. O.I. Falodun & O.A. Waleola, *Antibiotic resistance and virulence genes in Campylobacter species from pig and cattle samples in Ibadan, Nigeria.* **European Journal of Biological Research,** *14*(1), 2024, pg. 33-44.

16. L.M. Mamfe, C.A. Akwuobu, & E.O. Ngbede, *Phenotypic detection, antimicrobial susceptibility and virulence profile of staphylococci in the pig production setting, Makurdi, Nigeria,* **Access Microbiology,** *3*(12), 2021, pg. 000293.

17. O. O. Adebowale, F. A. Adeyemo, N. Bankole, M. Olasoju, H. K. Adesokan, O. Fasanmi, O. Adeyemo, O. Awoyomi, O. Kehinde, & F. O. Fasina, *Farmers' perceptions and*

drivers of antimicrobial use and abuse in commercial pig production, Ogun State, Nigeria, **International Journal of Environmental Research and Public Health,** *17*(10), 2020, pg. 3579.

18. I.A. Raufu, O.A. Ahmed, A. Aremu, J.A. Ameh, R.E. Timme, R.S. Hendriksen, & A.G. Ambali, *Occurrence, antimicrobial resistance and whole genome sequence analysis of Salmonella serovars from pig farms in Ilorin, North-central Nigeria,* **International Journal of Food Microbiology,** *350*, 2021, pg. 109245.

19. A.V. Kwaghe, D.E. Okomah, D.E. Aworh, E. Awosanya, C.D. Umeokonkwo, E. Yarai, C. Ameh, & J. Kabir, *Magnitude and trends of ruminants, Pigs and poultry diseases in Taraba State, Nigeria, 2013-2017: implications for public health.* **PAMJ-One Health,** *2*(20). 2020, pg. 11034

20. A.L.K. Abia, & S.Y. Essack, *Antimicrobial research and one health in Africa*. **Springer Nature,** 2023.

Chapter Two

Literature Review

2.1 Global Importance of Antibiotic Resistance

One of the most important worldwide health concerns of the twenty-first century is antibiotic resistance. Public health, food security, and economic stability are all seriously threatened by bacteria's resistance to the effects of antibiotics, which were formerly powerful tools against infectious diseases[1]. Antibiotic resistance is a serious and expanding public health issue, according to the World Health Organization (WHO), which also notes that it makes it more difficult to treat a wide variety of infections brought on by bacteria, parasites, viruses, and fungi. [2].

Antibiotic resistance is mostly caused by the overuse and abuse of antibiotics in both agriculture and human treatment. Antibiotics are frequently recommended in human medicine for illnesses like viral infections that don't require them or for which they don't work [3]. Antibiotics are widely utilized in agriculture as growth boosters and preventative measures for healthy animals in addition to treating illnesses 3. Because bacteria are constantly exposed to sub-lethal dosages of antibiotics due to their widespread use, resistant strains are more likely to survive due to selective pressure. [3].

Longer hospital stays, more medical expenses, and higher mortality rates are all consequences of antibiotic-resistant diseases. Over 2.8 million antibiotic-resistant illnesses occur in the United States annually, resulting in at least 35,000 fatalities, according to the Centers for Disease Control and Prevention (CDC) [3]. The problem is considerably worse on a global scale, with low- and middle-income nations bearing the brunt of the cost since they have less access to healthcare and effective antibiotics [4].

Antibiotic resistance has a significant economic impact. According to World Bank estimates, the ongoing increase in resistant infections may result in global economic harm comparable to that caused by the 2008 financial crisis by 2050 [4]. The loss of effective antibiotics would not only harm the treatment of infectious disorders but also weaken the efficacy of major surgeries and cancer treatments, which rely on good antibiotics to prevent and treat bacterial infections [4].

2.2 Overview of Multidrug-Resistant Bacteria

Bacterial strains that have developed resistance to several different kinds of antibiotics are known as multidrug-resistant (MDR) bacteria [6]. Because these bacteria can withstand the effects of several medications that are usually used to eradicate them, infections brought on by these pathogens are very challenging to treat [5]. Both horizontal gene transfer and genetic mutations are involved in the intricate and multidimensional processes by which bacteria acquire resistance genes [6].

Multidrug-resistant (MDR) bacteria can evade treatment through several distinct mechanisms. One such strategy involves the use of efflux pumps**,** which are specialized proteins that actively transport antibiotics out of the bacterial cell. By expelling these drugs, the bacteria reduce their intracellular concentration to levels that are no longer lethal [7]. Another mechanism is enzymatic degradation*,* where bacteria produce enzymes capable of chemically modifying or breaking down antibiotics, effectively neutralizing their action. A common example is the production of beta-lactamases**,** enzymes that dismantle beta-lactam antibiotics [6].

Bacteria may also develop resistance through target modification. In this process, they alter the structure of the molecules within their cells that antibiotics typically target, often through genetic mutations. These changes hinder the drug's ability to bind to its intended site, rendering it less

effective [7]. Additionally, some bacteria reduce their cell permeability by modifying their cell wall structures, thereby preventing antibiotics from penetrating the cell and reaching their targets [6].

Lastly, the formation of biofilms serves as a powerful protective mechanism. In this state, bacteria aggregate into dense communities surrounded by a self-produced matrix. This biofilm not only limits antibiotic penetration but also protects the bacteria from the host's immune defenses, further complicating treatment efforts. Together, these mechanisms make MDR bacteria particularly challenging to manage in clinical settings [8].

Common examples of MDR bacteria include Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococci (VRE), and numerous Gram-negative bacteria such as Escherichia coli and *Klebsiella pneumoniae* that manufacture extended-spectrum beta-lactamases (ESBLs) or carbapenemases. The prevalence of MDR bacteria is growing globally, driven by the causes mentioned above 5. The Pigs and other livestock can have MDR bacteria, which can subsequently spread to people through environmental pathways, the food chain, and direct contact. 5. This convergence of environmental, animal, and human health emphasizes how crucial a One Health strategy is to successfully and fully tackling the problem of antibiotic resistance.

Developing solutions to prevent the spread of multidrug resistance and preserve growth and spread of MDR bacteria in agriculture have been linked to the use of antibiotics in cattle. The effectiveness of currently available antibiotics requires an understanding of its causes and effects. [6.] This is especially crucial in areas like Ibadan, where pig farming is a major sector and where there is a substantial risk to public health from the establishment and spread of MDR bacteria.

2.3 Overview of Antibiotic Resistance

The effectiveness of antibiotics, which are necessary for treating bacterial infections in both humans and animals, is threatened by antibiotic resistance, a serious worldwide public health concern [1]. When bacteria develop defenses against the effects of antibiotics that were once successful in killing them or stopping their growth, this phenomenon takes place [7]. As a result, treating illnesses brought on by resistant bacteria gets harder, increasing morbidity, mortality, and medical expenses.

There are various ways that bacteria can develop resistance. The ability of some bacteria to naturally withstand specific drugs because of innate structural or functional traits is known as intrinsic resistance. [7]. Gram-negative bacteria, for example, have an outer barrier that can block the entry of antibiotics. Conversely, acquired resistance is brought about by genetic changes or horizontal gene transfer, which allows bacteria to acquire resistance genes from other bacteria [7]. Transduction, which involves the transfer of DNA by bacteriophages (viruses that infect bacteria); conjugation, which involves the direct contact of plasmids (small, circular DNA molecules) between bacteria; and transformation, in which bacteria absorb free DNA from the environment [8].

Antibiotic-resistant bacteria use specific mechanisms of action, such as the production of enzymes that chemically degrade or modify antibiotics (e.g., beta-lactamases breaking down beta-lactam antibiotics), the use of efflux pumps to expel antibiotics out of the cell before they can take effect, modification of the antibiotic's target site to decrease the drug's binding affinity and efficacy, and modifications to the bacterial cell wall or membrane to decrease antibiotic uptake [9].

Antibiotic resistance is caused by a number of factors, including the overuse and misuse of antibiotics in human medicine, agriculture, and aquaculture, which speeds up the selection of resistant bacteria; a lack of oversight and regulation in some areas, where antibiotics are available without a prescription, which makes the problem worse; poor infection control practices in healthcare settings, which allow the movement of people, animals, and goods across borders; and environmental factors, which include the possibility of antibiotics and resistant bacteria entering the environment through agricultural runoff, wastewater, and improper disposal, creating reservoirs of resistance [10].

Antibiotic resistance has far-reaching consequences. In terms of human health, resistant infections make diseases like sepsis, pneumonia, and tuberculosis more challenging to treat and result in lengthier hospital stays, more medical expenses, and higher fatality rates. In the field of veterinary medicine, resistant diseases can affect food safety, raise medical expenses, and decrease livestock productivity[10]. Antibiotic resistance has a financial impact on the world due to higher healthcare expenses, lost productivity, and the requirement for more costly treatments [10]. The accomplishments of modern medicine are undermined by antibiotic resistance, which also increases the risk of infections that are more difficult to treat during routine operations, cancer treatments, and organ transplants [11].

Antibiotic resistance is being addressed in a variety of ways. In order to minimize overuse and misuse of antibiotics in human and veterinary care, it is imperative to promote antibiotic stewardship. In order to track resistance trends, invest in comprehending resistance mechanisms, and create novel antibiotics and alternative treatments, surveillance and research are essential [12].

To stop the spread of resistant bacteria in healthcare and agricultural settings, strong infection prevention and control measures are required. To educate the public, legislators, and healthcare professionals about the dangers of antibiotic resistance and the significance of using antibiotics responsibly, public awareness and education campaigns are essential [12].

To effectively address this issue, international cooperation through agencies such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organization for Animal Health (OIE) is also essential. The problem of antibiotic resistance is intricate and multidimensional, necessitating consistent and coordinated efforts from various disciplines and sectors. To ensure the health of all it is imperative to adopt the One Health approach, which acknowledges the interdependence of environmental, animal, and human health [13].

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There are various ways that bacteria can develop resistance. The ability of some bacteria to naturally withstand specific antibiotics because of their innate structural or functional traits is known as intrinsic resistance[14]. Gram-negative bacteria, for example, have an outer membrane that prevents many antibiotics from working. Conversely, acquired resistance is brought about by genetic alterations or the horizontal transfer of resistance genes from other bacteria[15]. Transduction, which involves the transfer of DNA by bacteriophages (viruses that infect

bacteria); conjugation, which involves the direct contact of plasmids (small, circular DNA molecules) between bacteria; and transformation, in which bacteria absorb free DNA from the environment [15].

The production of enzymes that chemically break down or modify antibiotics (e.g., beta-lactamases breaking down beta-lactam antibiotics), changes to the antibiotic's target site to lower the drug's binding affinity, and modifications to the bacterial cell wall or membrane to decrease antibiotic uptake are some of the specific mechanisms of action that bacteria use to resist antibiotics [15].

Antibiotic resistance develops and spreads due to a number of variables. The selection of resistant bacteria is accelerated by the overuse and abuse of antibiotics in aquaculture, agriculture, and human medicine [15]. The issue is made worse in some areas where antibiotics are freely accessible due to a lack of control and regulation. While international travel and trade enable the movement of people, animals, and goods, which spreads resistant bacteria across borders16, inadequate infection control procedures in healthcare settings also contribute to the spread of resistant bacteria. Environmental considerations are particularly important since wastewater, inappropriate disposal, and agricultural runoff can all introduce antibiotics and resistant bacteria into the environment, resulting in the creation of reservoirs of resistance [16].

Antibiotic resistance has far-reaching consequences. In terms of human health, resistant infections make it more difficult to treat diseases like sepsis, pneumonia, and tuberculosis by increasing hospital stays, medical expenses, and mortality [17]. In the field of veterinary medicine, resistant diseases can affect food safety, raise medical expenses, and lower livestock productivity. Antibiotic resistance has a financial impact on the world due to higher healthcare expenses, lost productivity, and the requirement for more costly therapies [17]. The accomplishments of modern

medicine are undermined by antibiotic resistance, which also increases the risk of infections that are more difficult to treat during routine operations, cancer treatments, and organ transplants [17].

Antibiotic resistance is being addressed in a variety of ways. In order to minimize overuse and misuse of antibiotics in human and veterinary practice, it is imperative to promote antibiotic stewardship [18]. Research and surveillance are essential for tracking patterns of resistance, investing in the development of novel antibiotics and alternative treatments, and comprehending resistance processes [18]. To stop the spread of resistant bacteria, strong infection prevention and control strategies are required in both healthcare and agricultural contexts. To educate the public, legislators, and medical professionals about the dangers of antibiotic resistance and the significance of using antibiotics responsibly, public awareness and education campaigns are essential.

To effectively address this issue, international cooperation through agencies such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (OIE) is also essential [19]. The problem of antibiotic resistance is intricate and multidimensional, necessitating consistent and coordinated efforts from various disciplines and sectors. To ensure the health of all, it is imperative to adopt the One Health approach, which acknowledges the interdependence of environmental, animal, and human health.

2.4 Origin of the One Health Approach

A collaborative, multisectoral, and transdisciplinary approach, the One Health concept recognizes the interdependence of humans, animals, plants, and their shared environment in order to achieve optimal health results [19]. This theory emphasizes the interdependence of human, animal, and ecological health and the need for integrated solutions that cut across conventional

disciplinary lines to address problems like antibiotic resistance, zoonotic illnesses, and environmental degradation [20].

Understanding that many of the same viruses may infect both humans and animals and that the health of the environment has a direct impact on the health of all living things is the foundation of the One Health concept [21]. In order to address health risks at the animal-human-environment interface, this framework encourages cooperation between experts from a variety of fields, such as environmental science, public health, veterinary and human medicine, and policy-making [21].

The One Health concept is becoming more widely recognized as an essential foundation for addressing intricate and interrelated health issues. Antibiotic resistance, which is mostly caused by the abuse and overuse of antibiotics in human healthcare and agricultural practices, is one of the most urgent problems it tackles. It stops resistant bacteria from emerging and spreading throughout the human, animal, and environmental domains. The One Health viewpoint makes it feasible to create integrated strategies that

Prevention and management of zoonotic diseases diseases that are spread from animals to people is another crucial area in which One Health is essential. Surprisingly, more than 60% of infectious diseases that affect humans have animal roots. The One Health concept facilitates early detection, prompt reaction, and efficient control measures to reduce outbreaks of zoonotic diseases through cooperative efforts involving veterinary, medical, and environmental disciplines. The One Health concept also has a major positive impact on food security and safety. Coordinated actions across sectors are necessary to preserve the integrity of the food supply from production to consumption. In order to reduce contamination risks and advance public health,

One Health strengthens this by tying environmental protections, human health, and animal health together.

The strategy tackles general environmental health issues. Deforestation, pollution, and climate change are examples of human-caused changes that can upset ecosystems and have a detrimental effect on human and animal health. Since a healthy environment is essential to the wellbeing of all living things, the One Health approach places a strong emphasis on sustainable environmental activities. One Health provides a thorough and cooperative response to some of the biggest health risks of our day by bringing these disparate fields together.

2.5 Interconnection between Human, Animal, and Environmental Health

The interconnection between human, animal, and environmental health is central to the One Health approach. Pathogens do not recognize boundaries between species or environments. Zoonotic diseases, which can transfer between animals and humans, exemplify this connection. For instance, influenza viruses can originate in birds and pigs before being transmitted to humans. Similarly, diseases like rabies, Ebola, and Lyme disease highlight the direct health links between animals and humans [23].

The use of antibiotics in animals can lead to the development of resistant bacteria, which can then be transmitted to humans through direct contact, the food supply, or environmental pathways. For example, antibiotic-resistant Escherichia coli and Salmonella from livestock can contaminate meat products, water sources, and agricultural fields, posing significant public health risks [23].

Environmental factors such as water quality, soil health, and biodiversity play crucial roles in the health of humans and animals. Contaminated water can spread pathogens like Cryptosporidium and Giardia, affecting both livestock and human populations. Pesticides and other chemicals

used in agriculture can disrupt ecosystems, leading to increased disease transmission and antibiotic resistance [24].

The safety and security of food systems depend on the health of animals and the environment. Healthy livestock populations are essential for producing safe meat, dairy, and eggs. Sustainable agricultural practices that protect soil and water quality contribute to the overall health of ecosystems and the humans who rely on them for food [24].

Changes in climate impact the distribution and behavior of both pathogens and vectors (such as mosquitoes), altering the patterns of diseases. For example, warmer temperatures can expand the range of vector-borne diseases like malaria and dengue fever, affecting both human and animal populations [24].

The One Health approach emphasizes the need for integrated surveillance systems, shared data, and collaborative research to understand and mitigate these interconnected health risks. It advocates for joint health interventions, such as vaccination campaigns for animals to prevent zoonotic diseases, stewardship programs to reduce antibiotic use across sectors, and environmental conservation efforts to protect habitats and biodiversity [25]. By fostering collaboration and communication across disciplines, the One Health approach aims to create sustainable health solutions that benefit humans, animals, and the environment. This holistic perspective is crucial in a world where health challenges are increasingly complex and interrelated, and where isolated efforts are often insufficient to address the root causes of these issues [25].

2.6 Importance of Studying Pig Farms in Ibadan

Studying pig farms in Ibadan is crucial for several reasons, encompassing public health, agricultural sustainability, and socio-economic factors. Ibadan, a major city in Nigeria, is a significant agricultural hub where pig farming plays a vital role in the local economy. Understanding the dynamics of antibiotic use and the prevalence of multidrug-resistant (MDR) bacteria in this context is essential for multiple reasons[26].

Pigs can act as reservoirs for zoonotic pathogens, including MDR bacteria that can transfer to humans. Studying these farms helps identify potential public health risks associated with direct contact or consumption of contaminated pork products. The misuse and overuse of antibiotics in pig farming contribute to the development and spread of MDR bacteria. These resistant strains can compromise the effectiveness of antibiotics in treating human infections, posing a significant threat to public health [26].

Antibiotic resistance affects the health and productivity of livestock. Infections caused by resistant bacteria can lead to higher morbidity and mortality rates in pigs, negatively impacting farmers' livelihoods and food supply chains. The economic burden associated with managing antibiotic-resistant infections in livestock includes increased veterinary costs and potential trade restrictions. Understanding the extent of resistance can inform better management practices and policy decisions to ensure sustainable farming [27].

Antibiotic residues and resistant bacteria can enter the environment through manure, runoff, and improper disposal of animal waste. This environmental contamination can perpetuate the cycle of resistance, affecting ecosystems and potentially re-entering the food chain. Pig farming is a significant economic activity in Ibadan, providing income and employment for many local residents [27]. Ensuring the sustainability of this industry through proper management of antibiotic use and resistance is vital for the socio-economic stability of the region. Insights from studies on

antibiotic use and resistance in pig farms can inform local and national policies, helping to develop regulations that balance agricultural productivity with public health and environmental protection [28].

The use of antibiotics in agriculture dates back to the 1950s when they were first introduced to promote growth in livestock. Initially, antibiotics were hailed as a revolutionary tool that could prevent disease, enhance growth rates, and improve feed efficiency[23]. This era marked a significant transformation in livestock farming, enabling farmers to produce more meat, milk, and eggs with fewer resources. Low doses of antibiotics were added to animal feed to promote growth and improve feed conversion ratios. This practice led to increased productivity and economic gains[24].

Antibiotics were also used to prevent and treat bacterial infections in animals, reducing morbidity and mortality rates. The discovery of penicillin in 1928 by Alexander Fleming and subsequent antibiotics paved the way for their use in both human and veterinary medicine. Post-World War II, extensive research into antibiotics led to the development of various classes of antibiotics, including tetracyclines, macrolides, and sulfonamides, which were quickly adopted in agriculture [28]. The use of antibiotics in modern agriculture can be categorized into therapeutic, prophylactic, and sub-therapeutic applications. Each of these practices has distinct purposes and implications for animal health and antibiotic resistance [29]. Antibiotics are administered to animals diagnosed with bacterial infections. This practice is crucial for maintaining animal health and welfare, ensuring that sick animals receive appropriate medical treatment. Therapeutic doses are typically higher than those used for growth promotion or disease prevention, administered through injections, oral medications, or medicated feed and water [29].

Antibiotics are sometimes given to healthy animals to prevent the occurrence of diseases, particularly in high-density farming environments where the risk of disease transmission is high. This practice is common in intensive farming operations, where stress and close confinement increase the likelihood of infections [30].

Sub-therapeutic doses of antibiotics are added to animal feed to promote growth and improve feed efficiency. This practice has been widely criticized due to its contribution to the development of antibiotic resistance. Improved feed conversion rates result in faster growth and higher production yields, making sub-therapeutic use economically attractive for farmers [30].

Common antibiotics used includes tetracyclines which were widely used for their broad-spectrum activity and low cost. Macrolides are another type of antibiotics use to treat respiratory infections and promote growth in animals. Penicillins were effective against a wide range of bacterial infections while sulfonamides were commonly used in combination with other antibiotics to enhance efficacy [30].

The use of antibiotics in agriculture has both positive and negative impacts on animal health, influencing disease management, productivity, and resistance development. Antibiotics effectively control bacterial infections, reducing morbidity and mortality rates in livestock. Healthy animals exhibit better growth rates, higher productivity, and improved overall welfare [31]. Enhanced animal health and productivity translate to economic gains for farmers, contributing to food security.

Prolonged and indiscriminate use of antibiotics leads to the development of resistant bacterial strains, which can spread within animal populations. Antibiotic residues can persist in animal tissues, posing risks to consumers and potentially contributing to resistance in human pathogens

[31]. Antibiotics can disrupt the natural microbiome of animals, affecting digestion and immune function. Regulatory frameworks governing the use of antibiotics in agriculture vary globally, reflecting differences in public health priorities, agricultural practices, and levels of antibiotic resistance [31].

2.7 International Guidelines for the Use of Antibiotics in Agriculture

The WHO offers recommendations for the responsible use of antibiotics in animals raised for food, stressing the significance of reserving antibiotic usage for very necessary objectives. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) created Codex Alimentarius, an international food standards organization that establishes criteria for antibiotic residues in food [33]. The European Union (EU) has put strict rules into place, such as extensive surveillance programs for antibiotic resistance and a ban on the use of antibiotics for growth promotion that has been in place since 2006. Through the veterinarian Feed Directive (VFD), the U.S. Food and Drug Administration (FDA) has taken action to improve veterinarian control and gradually phase out the use of medically important antibiotics for growth promotion [32].

Regulatory frameworks in developing countries vary widely, with some countries lacking robust policies and enforcement mechanisms. Efforts are underway to harmonize regulations and improve antibiotic stewardship. The consequences of antibiotic use in agriculture extend beyond animal health, impacting human health, the environment, and global efforts to combat antibiotic resistance[33].

Resistant bacteria from animals can transfer to humans through direct contact, the food supply, and the environment. This poses a significant public health risk, as infections caused by MDR bacteria are difficult to treat. Residues of antibiotics in animal products can contribute to allergic

reactions and other health issues in consumers [34]. Strict monitoring and withdrawal periods are essential to ensure food safety. Antibiotics and resistant bacteria can enter the environment through animal waste, contaminating soil and water. This environmental contamination can perpetuate the cycle of resistance and affect wildlife. The widespread use of antibiotics can disrupt microbial communities in the environment, affecting nutrient cycling, soil fertility, and biodiversity [35].

Infections caused by MDR bacteria result in longer hospital stays, higher medical costs, and increased mortality. The economic burden extends to healthcare systems and families affected by resistant infections [36]. The loss of effective antibiotics could lead to higher disease rates in livestock, reduced productivity, and economic losses for farmers. Sustainable practices and alternative measures are needed to mitigate these risks [36]. Encouraging the judicious use of antibiotics through veterinary oversight and adherence to guidelines can reduce the development of resistance. Training farmers and veterinarians on the responsible use of antibiotics and resistance management is crucial for effective stewardship [37].

These supplements can promote gut health and enhance immune function in animals, reducing the need for antibiotics. Vaccinating animals against common infections can prevent disease outbreaks and decrease antibiotic use [37]. Enhancing biosecurity, hygiene, and animal welfare can reduce disease incidence and the reliance on antibiotics. Strengthening regulations and enforcement mechanisms can ensure the responsible use of antibiotics in agriculture. There is need to provide incentives for farmers to adopt best practices and alternative measures can promote sustainable agriculture [38].

Research into novel antimicrobial agents, vaccines, and non-antibiotic growth promoters is essential for reducing antibiotic dependence. Implementing robust surveillance systems to

monitor antibiotic use and resistance patterns can inform policy and guide interventions. Antibiotics have been essential to modern agriculture, but their overuse and misuse have contributed to the global problem of antibiotic resistance, which calls for a multifaceted approach that includes the prudent use of antibiotics, the adoption of alternative measures, strong regulatory frameworks, and ongoing research and innovation [39]. By adopting a One Health approach that acknowledges the interconnection between human, animal, and environmental health, we can work towards sustainable solutions that protect the efficacy of antibiotics and protect public health for future generations [40].

2.8 Multidrug-Resistant Bacteria in Livestock

Multidrug-resistant (MDR) bacteria in livestock represent a significant and growing concern globally. The prevalence of these bacteria in farm animals, including pigs, cattle, poultry, and other livestock, has been rising due to several factors [41]. The widespread use of antibiotics in animal husbandry for disease prevention, growth promotion, and therapeutic purposes has significantly contributed to this issue. In many regions, antibiotics are routinely administered to livestock, often without proper veterinary oversight, leading to the development and spread of resistant bacterial strains [42].

Studies have shown high levels of MDR bacteria in various livestock species. For instance, research conducted on pig farms frequently reports the presence of MDR pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, and multidrug-resistant Salmonella [42]. These pathogens are often resistant to multiple antibiotics, including those critically important for human medicine, such as cephalosporins, fluoroquinolones, and aminoglycosides [43].

The presence of MDR bacteria in livestock has several adverse effects on animal health. Infections caused by MDR bacteria are often more severe and difficult to treat, leading to higher rates of illness and death in affected animals. This impacts animal welfare and productivity, as sick animals grow slower and produce less milk, meat, or eggs [43]. The efficacy of commonly used antibiotics is compromised, necessitating the use of more expensive or less readily available alternatives. This not only increases treatment costs but also poses a challenge for veterinarians who must manage infections with limited therapeutic options [43].

The economic impact on the livestock industry is substantial. Increased veterinary care costs, reduced productivity, and potential trade restrictions on livestock products from farms with known MDR bacteria issues can lead to significant financial losses for farmers and the broader agricultural sector. The implications of MDR bacteria in livestock extend beyond animal health, posing significant risks to human health through several pathways [44].

Humans can contract MDR bacterial infections directly from animals through contact with infected livestock or indirectly through the consumption of contaminated meat, milk, or eggs. For instance, MDR Salmonella and Campylobacter species are common foodborne pathogens that can cause severe illness in humans. Antibiotic-resistant bacteria can spread from farms to the environment through runoff, manure, and wastewater [44]. These bacteria can contaminate soil, water sources, and crops, further perpetuating the cycle of resistance and increasing the risk of human exposure [44].

Antimicrobial resistance (AMR) in human pathogens is a problem that is exacerbated by the use of antibiotics in livestock. Bacteria that acquire resistance genes in animals can transfer these genes to human pathogens, making human infections more difficult to treat [45]. The interdependence of human, animal, and environmental health highlights the significance of a One

Health approach to combating MDR bacteria. Integrated efforts that address antibiotic use in agriculture, human medicine, and environmental management are crucial to halting the spread of resistance and safeguarding public health [45].

2.9 Addressing the Challenge of Resistance Due to Use of Antibiotics in Agriculture

Implementing stringent antibiotic use policies in agriculture to reduce unnecessary use and promote the responsible administration of these drugs. This includes improving veterinary oversight and promoting alternatives to antibiotics, such as vaccines and improved farm management practices. Enhancing surveillance systems to monitor the prevalence of MDR bacteria in livestock and the environment. This data is crucial for informing policy decisions and guiding targeted interventions[45].

Educating farmers, veterinarians, and the public about the risks of antibiotic resistance and the importance of responsible antibiotic use. Raising awareness can drive behavioral changes that reduce the spread of resistance [46]. Investing in research to develop new antibiotics, alternative therapies, and innovative farming practices that reduce reliance on antibiotics. This includes exploring the potential of probiotics, prebiotics, and other non-antibiotic strategies to maintain animal health [47].

Strengthening international cooperation to address the global nature of AMR. Organizations such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (OIE) play crucial roles in coordinating efforts and promoting best practices worldwide. Multidrug-resistant bacteria in livestock pose significant challenges to animal and human health [47]. Addressing this issue requires a comprehensive approach that integrates antibiotic stewardship, enhanced surveillance, public education, research,

and global collaboration. By adopting a One Health perspective, we can better understand and mitigate the risks associated with antibiotic resistance, ultimately protecting the health of animals, humans, and the environment [47].

2.10 The One Health Approach

A collaborative, multispectral, and transdisciplinary concept, the One Health approach acknowledges the interdependence of environmental, animal, and human health. This idea highlights the close connections between human, animal, and ecological health and the necessity of cross-sector collaboration in tackling health issues, especially those pertaining to infectious illnesses and antibiotic resistance [72].

The idea of One Health has developed over many years, becoming more well-known as global health issues brought attention to the connections between environmental, animal, and human health. The necessity of a comprehensive approach to health has been highlighted by the discovery of zoonotic diseases, or illnesses spread from animals to people, like COVID-19, Ebola, and H1N1 influenza. Although its tenets extend back to the 19th century, when veterinary medicine first acknowledged the connection between human and animal health, the phrase "One Health" was publicly adopted in the early 2000s[72].

2.11 Core Principles of One Health

Interdisciplinary Collaboration: One Health emphasizes the need for cooperation among various disciplines, including medicine, veterinary science, ecology, microbiology, and public health. This collaboration helps in understanding and addressing health issues from multiple perspectives [73].

Holistic Approach: The approach advocates for considering all aspects of health—human, animal, and environmental—when developing health policies and interventions. This holistic view ensures comprehensive strategies that address root causes rather than just symptoms [74].

Preventive Measures: One Health focuses on prevention, emphasizing the importance of surveillance, early detection, and control of health threats at their source. This proactive stance helps in mitigating potential outbreaks and health crises [75].

Sustainable Solutions: Sustainable practices are integral to One Health, promoting the balance between economic development, environmental preservation, and health improvement. Sustainable agricultural practices, wildlife conservation, and pollution control are examples of this principle in action [76].

2.12 Applications of One Health

1. Combating Zoonotic Diseases

Zoonotic diseases are a primary focus of the One Health approach. Approximately 75% of emerging infectious diseases in humans are zoonotic in origin. By monitoring and controlling diseases in animals, One Health aims to prevent their transmission to humans. For example, the surveillance of avian influenza in poultry can help prevent outbreaks in humans [77].

2. Addressing Antimicrobial Resistance (AMR)

Antimicrobial resistance is a serious problem that affects the health of people, animals, and the environment. The creation of resistant bacteria is a result of the overuse and abuse of antibiotics in agriculture, animals, and humans. One Health supports the research of new antimicrobials and substitutes as well as the prudent use of antibiotics in all fields [78].

3. Ensuring Food Safety and Security

By keeping an eye on and managing foodborne diseases, One Health plays a critical role in guaranteeing food safety and security. The entire food production process—from farm to fork—is covered by the method. This guarantees that food items are safe to eat and that contamination risks are kept to a minimum [78].

4. Environmental Health

Environmental health is a crucial component of the One Health approach. Pollution, climate change, and habitat destruction have direct and indirect impacts on human and animal health. One Health advocates for sustainable environmental practices, such as reducing pollution and protecting natural habitats, to promote overall health [78].

2.13 Applications of One Health in Controlling Antibiotic Resistance

The complicated problem of antibiotic resistance (AR), which is a serious danger to agriculture, the environment, and world health, is best addressed via the One Health strategy. Understanding how human, animal, and environmental health are intertwined, One Health advocates for coordinated approaches to address antibiotic resistance [79].

1. Antibiotic Stewardship Programs

To guarantee the ethical use of antibiotics, One Health supports antibiotic stewardship initiatives in animal medicine, agribusiness, and human healthcare. Putting into practice recommendations for the proper prescription of antibiotics, informing patients and healthcare professionals about the dangers of abuse and overuse, and keeping an eye on trends in antibiotic usage and resistance [7].

Establishing rules for the responsible use of antibiotics in animals, encouraging probiotics and vaccinations as antibiotic substitutes, and making sure that prescriptions for antibiotics are supervised by veterinarians. Lowering the amount of antibiotics used to promote livestock development and prevent disease, putting biosecurity measures in place to stop infections, and encouraging appropriate husbandry techniques to lessen the need for drugs [79].

Surveillance and Monitoring

Effective surveillance and monitoring systems are essential for tracking antibiotic resistance patterns and guiding interventions [79].

Integrated Surveillance Systems

Developing and maintaining integrated surveillance systems that collect data on antibiotic use and resistance from human healthcare, veterinary practices, and agricultural settings. This allows for a comprehensive understanding of resistance trends and hotspots [80].

Global Collaboration

Encouraging global cooperation and data exchange via agencies such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (OIE). This aids in coordinating response activities and monitoring the global spread of resistance [80].

3. Research and Development

Finding novel ways to combat antibiotic resistance requires funding research and development. Funding studies to create novel antibiotics and complementary therapies including natural products, bacteriophages, and antimicrobial peptides. These substitutes can lessen the need for

conventional antibiotics. Establishing rewards for adherence to legislation and antibiotic stewardship initiatives 81. Financial rewards for farmers who adopt best practices and sanctions for non-compliance are two examples of this. One important element of the One Health strategy is addressing the environmental causes of antibiotic resistance. Putting in place appropriate waste management procedures to stop the discharge of resistant microorganisms and antibiotics into the environment. This involves cleaning up wastewater from farms, hospitals, and pharmaceutical manufacturing facilities[82].

2.14 Case Studies and Examples

One of the best examples of One Health ideas being successfully used to the management of antibiotic resistance is Denmark. The nation put in place a comprehensive program that improved farm management techniques, promoted alternatives like probiotics and immunizations, and imposed stringent controls on the use of antibiotics in cattle. Denmark witnessed a matching decline in antibiotic resistance 46 as a result of drastically reducing the usage of antibiotics in cattle.

NethMap/MARAN is an integrated surveillance system created in the Netherlands that tracks antibiotic usage and resistance in food, animals, and people. Important data from this system is used to guide governmental interventions and policy. The joint endeavor is an example of the One Health strategy and spans several sectors, including agriculture, veterinary care, and public health. Several future directions are needed to improve the One Health approach to antibiotic resistance reduction.

Strengthening global coordination and cooperation through agencies such as WHO, FAO, and OIE to address antibiotic resistance. Putting money into cutting-edge technologies for quick antibiotic resistance detection, monitoring, and surveillance [75]. Creating all-encompassing One

Health laws that combine environmental, animal, and human health regulations to combat antibiotic resistance in a comprehensive manner. Using participatory methods that take into account local knowledge and customs to involve communities in attempts to combat antibiotic resistance [75]. A thorough framework for dealing with the complex problem of antibiotic resistance is offered by the One Health approach. Through encouraging research and development, education, integrated surveillance, interdisciplinary cooperation, and robust policies. This approach can significantly contribute to the control and prevention of antibiotic resistance. Adopting this strategy is necessary to safeguard the environment, human health, and animal health, as well as to ensure a sustainable and healthy future[74].

2.15 Evolution of Antibiotic Use in Agriculture

The use of antibiotics in agriculture has a complex history, characterized by significant milestones that have shaped current practices and policies. This section explores the evolution of antibiotic use in agriculture, highlighting key developments, scientific discoveries, and the resulting impacts on animal health, agricultural productivity, and public health [47].The discovery of penicillin by Alexander Fleming in 1928 marked the beginning of the antibiotic era. Initially used to treat bacterial infections in humans, its success led to the search for other antibiotics. Following World War II, scientists began exploring the use of antibiotics in livestock. The first notable use was to control diseases in poultry and livestock, leading to improved animal health and productivity [48].

By the 1950s, antibiotics such as tetracyclines, penicillins, and sulfonamides were introduced into animal husbandry. Researchers discovered that these drugs could be added to animal feed at sub-therapeutic levels to promote growth and enhance feed efficiency. Farmers observed significant improvements in growth rates and feed conversion ratios, making antibiotics an

integral part of modern agricultural practices. Antibiotics became widely adopted as growth promoters, particularly in intensive farming operations [49]. This practice was based on the premise that antibiotics could improve nutrient absorption and reduce subclinical infections, leading to healthier and more productive animals.

Antibiotics were also used prophylactically to prevent outbreaks of bacterial infections in densely populated farming environments. Initially, there were few regulations governing antibiotic use in agriculture. However, as antibiotic resistance began to emerge, regulatory agencies started to take notice. In the United States, the Food and Drug Administration (FDA) began approving antibiotics for use in livestock, setting guidelines for their administration and residue limits in animal products [49].

Scientists began to notice trends of antibiotic resistance in veterinary and human medicine during the 1960s and 1970s. Research has shown that horizontal gene transfer and genetic changes can cause bacteria to become resistant to antibiotics. Public health concerns were highlighted by the connection between the use of antibiotics in agriculture and the emergence of resistant bacterial strains. Researchers cautioned that environmental pathways, dietary sources, and direct contact could all be ways for resistant bacteria to spread from animals to people [50].

he UK's Swann Report from 1969 suggested limiting the use of antibiotics in animals to medicinal uses under a veterinarian's supervision. One of the earliest reports to draw attention to the dangers of indiscriminate antibiotic use in agriculture was this one [47]. To reduce the dangers of antibiotic resistance, the World Health Organization (WHO) and other international organizations started releasing recommendations and guidelines. One of these was restricting the use of antibiotics that are crucial for medicine in agriculture. Significant action was taken by the EU to reduce the use of antibiotics as growth promoters [47].

Changes in agricultural practices among member states resulted from the EU's 2006 prohibition on the use of antibiotics in animal feed for growth enhancement. In 2015, the FDA unveiled the Veterinary Feed Directive (VFD), which mandates veterinary supervision for the use of antibiotics that are medically significant in animal feed and water[50].

Guidelines for antibiotic residues in food are among the international food standards produced by the Codex Alimentarius Commission, which was formed by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [52]. The One Health method gained popularity as a result of the realization that the health of humans, animals, and the environment are interconnected. By using integrated tactics that involve different sectors, this holistic approach seeks to reduce antibiotic resistance. Using a One Health strategy, the identification and isolation of multidrug-resistant bacteria from pig farms in Ibadan, Nigeria, can be intricately linked to local and international regulatory rules, as well as compliance and enforcement concerns [53].

Action Plans for Antimicrobial Resistance (AMR): Antimicrobial resistance is a serious issue linked to the widespread use of antibiotics in agriculture, and international organizations such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have created action plans to combat it. These plans place a strong emphasis on the value of surveillance, the prudent use of antibiotics, and the creation of substitute tactics[54].

Regulations governing international trade frequently contain safeguards against the spread of infectious diseases, such as those brought on by bacteria resistant to antibiotics. This can involve requirements for veterinary health certificates, sanitary standards for livestock products, and restrictions on the importation of certain animal products [54]. Animal Health and Agriculture Regulations: Local governments typically have regulations governing animal husbandry

practices, including the use of antibiotics in livestock farming. Compliance with these regulations may involve restrictions on the types and doses of antibiotics used, as well as requirements for veterinary oversight and reporting of antibiotic use [55].

Local public health authorities may implement regulations to prevent the spread of infectious diseases from animals to humans (zoonoses), including those caused by multidrug-resistant bacteria. This could involve measures such as surveillance of antibiotic-resistant pathogens, quarantine protocols, and public education campaigns on hygiene and food safety [55].

2.16 Compliance and Enforcement Issues in the Use of Antibiotics in Agriculture

a. Lack of Regulatory Compliance: One of the key challenges is ensuring compliance with existing regulations governing antibiotic use in agriculture. This may be due to factors such as limited resources for regulatory oversight, inadequate enforcement mechanisms, or resistance from stakeholders within the agricultural industry [56].

b. Weak Surveillance Systems: Effective surveillance is crucial for detecting and monitoring the emergence of multidrug-resistant bacteria on pig farms. However, many regions, particularly in low- and middle-income countries, face challenges in establishing robust surveillance systems due to limitations in laboratory capacity, data collection, and reporting infrastructure [56].

c. Cross-Sectoral Coordination: Addressing the issue of multidrug-resistant bacteria requires collaboration across multiple sectors, including human health, animal health, agriculture, and environmental management. Achieving effective coordination and cooperation between these sectors can be challenging due to differences in priorities, mandates, and stakeholder interests [57].

The isolation and characterization of multidrug-resistant bacteria from pig farms in Ibadan, Nigeria, within a One Health approach, necessitates alignment with both global and local

regulatory policies, along with efforts to address compliance and enforcement issues to mitigate the spread of antimicrobial resistance [57].

2.17 Multidrug-Resistant Bacteria in Pig Farms

Pig farms represent a unique environment where the selective pressure of antimicrobial use promotes the emergence and spread of multidrug-resistant bacteria. The factors contributing to the prevalence of resistant pathogens in pig farming, including overcrowding, poor sanitation, and suboptimal management practices. Case studies and research findings illustrate the extent of antimicrobial resistance in pig farms worldwide, highlighting the urgent need for intervention [58].

Despite the existence of regulatory policies, compliance with antibiotic use guidelines remains a significant challenge in pig farming. Factors contributing to poor compliance include economic incentives for antimicrobial use, lack of awareness among farmers, and inadequate veterinary oversight [58]. Effective enforcement of antibiotic use regulations requires robust surveillance systems capable of monitoring antimicrobial consumption, detecting resistant pathogens, and tracking trends over time.

The One Health approach emphasizes the interconnectedness of human, animal, and environmental health in addressing complex health challenges such as antimicrobial resistance. We explore the role of interdisciplinary collaboration in designing holistic interventions that target antimicrobial use in pig farming while considering broader ecological and socioeconomic factors. Case studies highlight successful One Health initiatives aimed at reducing antimicrobial resistance in agricultural settings [58]. These recommendations include strengthening regulatory oversight, promoting responsible antibiotic use practices, investing in surveillance infrastructure, and fostering cross-sectoral collaboration through the One Health approach [59].

Multidrug-resistant bacteria in pig farms present a multifaceted challenge that requires a comprehensive and coordinated response from policymakers, regulators, industry stakeholders, and the broader public. By adopting a One Health approach and addressing regulatory policies, compliance issues, and enforcement challenges, we can work towards mitigating the spread of antimicrobial resistance and safeguarding human, animal, and environmental health for future generations [59].

2.18 Current Trends in Pig Farming

Modern agricultural practices increasingly emphasize antibiotic stewardship, promoting the judicious use of antibiotics and exploring alternatives such as probiotics, prebiotics, and improved husbandry practices. Enhanced surveillance systems monitor antibiotic use and resistance patterns, informing policy decisions and guiding interventions to mitigate resistance [60]. Ongoing research aims to develop new antimicrobial agents, vaccines, and non-antibiotic growth promoters. Innovations in precision farming and biotechnology also hold promise for reducing reliance on antibiotics [60].

International collaboration and harmonization of regulations are essential for addressing antibiotic resistance. Efforts focus on creating a global framework for responsible antibiotic use and resistance management. The evolution of antibiotic use in agriculture reflects a journey from the initial discovery and widespread adoption of antibiotics to the growing awareness of antibiotic resistance and the subsequent regulatory responses [59].

2.19 Common Antibiotics Used in Pig Farming

Antibiotics are essential tools in pig farming for maintaining animal health, ensuring productivity, and preventing disease outbreaks. Various classes of antibiotics are commonly used, each with

specific indications, benefits, and risks. Below are some of the most frequently used antibiotics in pig farming:

a.Tetracyclines

Tetracyclines, such as oxytetracycline and chlortetracycline, are broad-spectrum antibiotics widely used in pig farming. They are effective against a range of bacterial infections including respiratory diseases, enteric infections, and systemic infections. They are commonly administered through feed and water but can also be given via injection for severe infections. Tetracyclines are valued for their cost-effectiveness and broad spectrum of activity. Concerns include the overuse can lead to resistance, reducing their effectiveness over time[47].

b. Macrolides

Macrolides, such as tylosin and erythromycin, are used primarily to treat respiratory infections caused by Mycoplasma, Pasteurella, and other pathogens. They are also effective against some enteric diseases. Typically administered through feed or water, and less frequently via injection. Macrolides are effective against specific pathogens resistant to other antibiotics and have anti-inflammatory properties. Resistance can develop, and there are concerns about their use due to similarities with human antibiotics [49].

c. Penicillins

Penicillins, including amoxicillin and penicillin G, are used to treat a variety of bacterial infections such as erysipelas, pneumonia, and streptococcal infections. Penicillinc can be administered via injection for immediate and effective action, as well as through feed and water for mass treatment. Penicillins are highly effective against Gram-positive bacteria and have a long history of use. Resistance is a significant issue, particularly due to their extensive use [49].

d. Sulfonamides

Sulfonamides, such as sulfamethazine and trimethoprim-sulfa combinations, are used to treat respiratory and enteric infections, as well as systemic infections. It administered through feed and water, and sometimes via injection. It is also often used in combination with other antibiotics to enhance efficacy. Resistance development is a notable concern, necessitating careful management [50].

e. Aminoglycosides

Aminoglycosides, like gentamicin and neomycin, are used for their efficacy against Gram-negative bacteria, particularly in neonatal piglets for enteric infections. They are administered via injection and sometimes topically for localized infections. Effective against a wide range of bacteria, particularly those resistant to other antibiotics. Potential for nephrotoxicity and ototoxicity, and strict withdrawal periods are required to ensure food safety [50].

f. Fluoroquinolones

Fluoroquinolones, such as enrofloxacin and marbofloxacin, are used to treat respiratory infections and other systemic infections. Primarily administered via injection for targeted treatment. Broad-spectrum activity and high efficacy. Restrictions on use due to significant resistance concerns and their importance in human medicine [50]. The administration of antibiotics in pig farming is tailored to ensure effective delivery, compliance with regulations, and minimal stress to animals. The three primary methods of administration are through feed, water, and injection. Antibiotics are mixed into the feed, allowing for mass medication of the herd. This method is convenient and ensures consistent dosing over time [50]. Commonly used for growth promotion (where allowed), disease prevention, and treatment of chronic conditions. Ease of

administration, especially for large herds, and minimal handling stress for animals. Inconsistent intake due to variations in individual animal feeding behavior and the potential for sub-therapeutic dosing leading to resistance [50].

2.20 Methods for Isolation and Characterization of MDR Bacteria

Isolating multidrug-resistant (MDR) bacteria involves several meticulous steps to ensure accurate identification and further study. These steps typically begin with the collection of samples from various environments, followed by selective culturing and preliminary identification of bacterial isolates.

1. Sample Collection

Samples for isolation of MDR bacteria can be collected from various sources including animal feces, soil, water, and surfaces in farm environments. Each sample type may require specific collection tools and conditions to prevent contamination. Samples should be transported in sterile containers and maintained at appropriate temperatures to preserve bacterial viability until processing. Proper labeling and documentation are crucial for traceability and accurate results [45].

2. Selective Culturing

Selective and differential media are crucial for isolating specific bacteria from mixed populations. For example, MacConkey agar is used to isolate Gram-negative bacteria, while Mannitol Salt Agar (MSA) is used for Gram-positive bacteria like *Staphylococcus aureus*. These media contain substances that inhibit the growth of non-target organisms, allowing for the selective growth of target bacteria. In other isolate bacteria, media containing antibiotics can be employed. Only bacteria that are resistant to the antibiotics present in the media will grow, thus selecting for strains[47]. Proper incubation conditions such as temperature, humidity, and atmospheric

requirements (aerobic or anaerobic) are critical for the growth of bacteria. Typically, incubation temperatures range from 35-37°C, depending on the specific bacterial species being targeted [49].

3. Preliminary Identification

Colony morphology is observed for characteristics like shape, size, color, and hemolytic activity on blood agar can provide initial clues about the bacterial species. Gram staining technique is also done which is a fundamental technique that differentiates bacteria into Gram-positive and Gram-negative groups based on cell wall properties, aiding in further identification steps and guiding the selection of appropriate media for sub-culturing [51].

4. Characterization Methods Including Molecular Techniques

After isolating MDR bacteria, it is essential to characterize them to understand their resistance profiles, species, and the genetic mechanisms underpinning their resistance. This involves both phenotypic and molecular methods.

a. Phenotypic Characterization: Biochemical tests such as catalase, oxidase, coagulase, and carbohydrate fermentation profiles are employed to identify bacterial species. These tests rely on the metabolic properties of bacteria and can be conducted using commercially available kits [451].

b. **Antibiotic Susceptibility Testing (AST):** Methods like disk diffusion (Kirby-Bauer), broth microdilution, and E-test are used to determine the susceptibility of bacterial isolates to various antibiotics. The results categorize bacteria as susceptible, intermediate, or resistant based on clinical breakpoints established by organizations like the Clinical and Laboratory Standards Institute (CLSI) [51].

2. **Molecular Techniques**

a. Polymerase Chain Reaction (PCR): PCR is used to amplify specific DNA sequences to detect the presence of resistance genes. For instance, the mecA gene in *Staphylococcus aureus* indicates methicillin resistance[51].

b. Multiplex PCR: This variant of PCR allows the simultaneous amplification of multiple target genes in a single reaction, enabling the detection of several resistance genes at once.

c. Real-Time PCR (qPCR): qPCR not only amplifies DNA but also quantifies it, providing information about the abundance of resistance genes and allowing for the study of gene expression levels

3. Genomic Techniques

a. Whole Genome Sequencing (WGS): WGS provides a comprehensive analysis of the bacterial genome, identifying all resistance genes, mutations, and mobile genetic elements. It also helps in understanding the evolutionary relationships between strains [52].

b. Sequencing of Specific Genes: Sequencing specific genes, such as the 16S rRNA gene for bacterial identification or resistance genes, provides precise information on species and resistance mechanisms [52].

4. Proteomic and Metabolomic Approaches

a.**Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry:** This technique profiles the protein composition of bacterial cells, aiding in rapid identification and characterization. It can also detect specific protein markers associated with antibiotic resistance [52].

b. **Metabolomics:** Analyzing the metabolic profiles of bacteria can reveal insights into their physiological state and resistance mechanisms.

5. Phenotypic Microarrays

a. Biofilm Formation Assays: These assays measure the ability of bacteria to form biofilms, which are associated with increased resistance to antibiotics.

b. Efflux Pump Activity Assays: These assays assess the activity of efflux pumps, which are mechanisms that bacteria use to expel antibiotics from their cells, contributing to resistance.

6. Advanced Imaging Techniques

a. Fluorescence Microscopy: This technique is used to study the localization and expression of resistance proteins within bacterial cells.

b. Electron Microscopy: This technique provides detailed images of bacterial cell structures, helping visualize changes associated with resistance mechanisms.

2.21 Benefits of Integrated Surveillance Systems

Early identification of emerging resistance patterns allows for prompt intervention, reducing the spread of MDR bacteria. Comprehensive data enables stakeholders to make informed decisions regarding antimicrobial stewardship and infection control measures. These systems foster collaboration and information exchange among sectors, enhancing the overall effectiveness of surveillance and control efforts [48]. The One Health approach underscores the necessity of surveillance across human health, animal health, and environmental sectors to effectively tackle the issue of antimicrobial resistance [48]. The One Health approach advocates for a collaborative,

multi-sectoral, and transdisciplinary strategy to achieve optimal health outcomes. It recognizes that human health, animal health, and ecosystem health are inextricably linked [51].

2.22 Benefits of Cross-Sectorial Surveillance

Surveillance across sectors provides a holistic view of the dynamics of MDR bacteria, including sources and transmission pathways. Cross-sectorial surveillance enhances the ability to prevent and control MDR bacterial infections in both humans and animals. This dual benefit protects public health and reduces the burden of antibiotic-resistant infections [48].

By coordinating efforts and sharing infrastructure, resources are used more efficiently. This reduces duplication of efforts and fosters synergy among different sectors, making surveillance and intervention efforts more cost-effective [78]. Data from integrated surveillance inform the creation of evidence-based policies and regulations for antimicrobial use. These policies are critical in guiding practices that minimize the development and spread of resistance [77].

Effective surveillance systems contribute to global efforts to combat antimicrobial resistance, ensuring alignment with international standards and guidelines. This is vital for maintaining global health security and addressing transboundary health threats [73]. The integration of surveillance systems and the emphasis on cross-sectoral surveillance are pivotal in managing the threat posed by MDR bacteria. The One Health approach ensures a comprehensive, coordinated response that enhances the health and well-being of humans, animals, and the environment [72]. By leveraging the strengths of integrated surveillance systems, stakeholders can implement more effective strategies to combat antimicrobial resistance and safeguard public health [72]

2.23 Successful One Health Initiatives Globally

The One Health approach recognizes the interconnectedness of human, animal, and environmental health and promotes collaborative efforts across these domains to address health threats. This approach has been instrumental in several successful initiatives globally, particularly in combating antimicrobial resistance and zoonotic diseases.

1. The Global Health Security Agenda (GHSA)

The GHSA is an international partnership launched in 2014 aimed at strengthening global capacity to prevent, detect, and respond to infectious disease threats. By emphasizing the One Health approach, GHSA fosters collaboration across human, animal, and environmental health sectors. Notable achievements include improving laboratory capacity, enhancing surveillance systems, and bolstering workforce development in partner countries [74]. GHSA has played a crucial role in mitigating the risks of zoonotic diseases and antimicrobial resistance through coordinated global efforts [74].

2. USAID's Emerging Pandemic Threats (EPT) Program

The EPT Program, initiated by the United States Agency for International Development (USAID), focuses on identifying and mitigating pandemic threats at their source. The program utilizes a One Health framework to enhance surveillance and response capabilities in regions prone to zoonotic spillover [58].

3. The Tripartite Alliance (FAO, OIE, WHO)

To address health concerns at the human-animal-environment interface, the Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), and the World Health Organization (WHO) have formed the Tripartite Alliance [52]. This partnership has been essential in the fight against zoonotic illnesses like rabies and avian influenza [52]. The Tripartite Alliance promotes coordinated response plans, integrated surveillance, and shared risk assessments to improve global health security and support One Health policy implementation.

4. The Global Antimicrobial Resistance Surveillance System (GLASS)

GLASS was started by WHO with the goal of strengthening international efforts to track and fight antimicrobial resistance (AMR). By combining information from the fields of environmental, animal, and human health, GLASS uses a One Health approach [78]. The creation of focused therapies is guided by the insightful information this extensive surveillance system offers about the spread of AMR. The global ability to monitor and address AMR risks has improved as a result of GLASS's effective expansion of involvement to many nations 78.

5. The Zoonoses and Emerging Livestock Systems (ZELS) Program

The ZELS Program, funded by the United Kingdom government, focuses on understanding and mitigating zoonotic disease risks in livestock systems [73]. By adopting a One Health perspective, ZELS conducts interdisciplinary research to explore the drivers of zoonotic disease transmission and develop effective control strategies. Successful projects under ZELS have improved biosecurity practices, enhanced disease surveillance, and promoted sustainable livestock management in several low- and middle-income countries [73].

6. The One Health Workforce (OHW) Project

The OHW Project, supported by USAID, aims to build a skilled workforce capable of addressing One Health challenges [52]. This initiative collaborates with universities and institutions in Africa and Southeast Asia to develop and implement training programs in One Health competencies. The OHW Project has successfully trained thousands of professionals in interdisciplinary approaches to disease prevention and control, fostering a resilient workforce ready to tackle emerging health threats [52].

These six initiatives exemplify the success of the One Health approach in addressing complex health challenges at the human-animal-environment interface. By promoting integrated surveillance systems, enhancing cross-sectorial collaboration, and building capacity, these initiatives have significantly contributed to global health security. The lessons learned from these successful programs underscore the importance of continued investment in One Health strategies to safeguard public health and combat emerging health threats [51].

2.24 Challenges and Opportunities: Barriers to Implementation of One Health Initiatives

Addressing health risks that cut across the human, animal, and environmental realms requires the One Health approach. Although this strategy has a lot of potential, there are several obstacles and opportunities in putting One Health efforts into practice. Promoting efficient cooperation between experts from many disciplines, such as public health, veterinary medicine, environmental science, and medicine, is one of the main obstacles[78].

Establishing interdisciplinary training programs and joint research projects can promote mutual understanding and collaboration. Encouraging cross-disciplinary education at the academic level can also lay the groundwork for future cooperation. One Health initiatives often require substantial financial resources and sustained investment. Securing funding can be challenging

due to competition with other health priorities and the complexity of coordinating across sectors [74].

Advocating for the cost-effectiveness of One Health interventions, which can prevent costly outbreaks and long-term health issues, can help attract funding. International organizations and partnerships can pool resources to support One Health projects [72]. The implementation of One Health initiatives is complicated by differing regulatory frameworks and policies across sectors and countries. Lack of political will and coordination can impede the establishment of integrated surveillance and response systems. Developing unified policies and regulatory frameworks that encourage cross-sectoral collaboration can facilitate One Health initiatives. Engaging policymakers through evidence-based advocacy can enhance political support for these initiatives [42].

Effective One Health surveillance requires seamless data sharing across sectors, which is often hindered by data silos, privacy concerns, and lack of standardized data collection methods. Creating interoperable data platforms and standardized protocols can improve data sharing and integration. Promoting open data policies while ensuring data privacy can facilitate the flow of information across sectors [42].

Cultural differences and social norms can affect the acceptance and implementation of One Health initiatives [40]. In some communities, traditional beliefs about health and disease may conflict with scientific approaches. Engaging community leaders and stakeholders in the design and implementation of One Health programs can enhance acceptance. Culturally sensitive education and communication strategies can bridge gaps between scientific and traditional perspectives [41]. There is often a shortage of professionals trained in One Health approaches, limiting the capacity to implement and sustain initiatives. This is particularly acute in low- and

middle-income countries. Investing in education and training programs to build a skilled One Health workforce is crucial. International collaborations can provide training opportunities and technical assistance to build local capacity [41].

Integrating human, animal, and environmental health surveillance systems can provide a comprehensive understanding of disease dynamics. This can lead to early detection and more effective responses to outbreaks [41]. One Health initiatives can lead to better health outcomes by addressing the root causes of diseases that affect multiple sectors [41]. This holistic approach can prevent the spread of zoonotic diseases and improve overall public health. Preventing and controlling diseases through a One Health approach can be more cost-effective than dealing with the consequences of outbreaks. Coordinated efforts can reduce duplication of resources and improve efficiency [42]. One Health initiatives enhance global health security by addressing transboundary health threats. International collaboration and standardized approaches can improve preparedness and response to pandemics [42].

By promoting healthy ecosystems, One Health initiatives contribute to sustainable development goals. Addressing the environmental determinants of health can lead to more resilient and sustainable communities. The implementation of One Health initiatives faces significant challenges, including interdisciplinary collaboration, funding, policy coordination, data sharing, cultural barriers, and human resource capacity [43]. However, these challenges also present opportunities for innovation and improvement [43]. By investing in education, fostering collaboration, and advocating for integrated policies and funding, stakeholders can overcome these barriers and realize the full potential of the One Health approach. Successfully implementing One Health initiatives can lead to enhanced disease surveillance, improved public

health outcomes, cost savings, strengthened global health security, and sustainable development [43].

2.25 Interventions and Control Measures in One Health Initiatives

The One Health approach advocates for comprehensive interventions and control measures that address health threats at the intersection of human, animal, and environmental health. Effective implementation of One Health initiatives involves coordinated actions across various sectors to prevent and control diseases [44].

2.25.1 Integrated Surveillance Systems

Implement standardized protocols for collecting data on diseases and antimicrobial resistance from humans, animals, and the environment. Develop interoperable databases that allow for real-time data sharing and analysis across sectors [43]. Establish systems that can detect and respond to emerging health threats promptly, leveraging data from multiple sources. Regular monitoring of zoonotic diseases and antimicrobial resistance trends. Rapid response teams to investigate and contain outbreaks in both human and animal populations [45].

There is a need to develop and disseminate evidence-based guidelines for the appropriate use of antimicrobials in healthcare, veterinary, and agricultural settings. There is also need to provide ongoing education and training for healthcare providers, veterinarians, and farmers on antimicrobial stewardship practices. Scientist must monitor antimicrobial prescriptions and usage patterns to ensure compliance with guidelines [45].

2.25.2 Control Measures for Use of Antibiotics

There is the need for regulation of antimicrobial Sales. There must be the implementation of policies to regulate the sale and distribution of antimicrobials. There is need to encourage the use of alternative measures such as vaccination, improved hygiene, and biosecurity to reduce the need for antimicrobials [46]. Biosecurity measures must also be put in place to prevent the introduction and spread of infectious agents within and between populations of humans and animals. There is need to develop and implement biosecurity plans for farms, including measures such as quarantine protocols, disinfection practices, and controlled access to animal facilities [47].

Monitor wildlife is also important for emerging infectious diseases that could spill over into human and domestic animal populations. There is need to implement measures to control vectors (e.g., mosquitoes, ticks) that transmit zoonotic diseases [47]. Hygiene and sanitation must be promoted especially hygiene and sanitation practices in human and animal health settings to reduce the risk of pathogen transmission. There is also the need to implement vaccination programs for livestock, pets, and wildlife to prevent the spread of infectious diseases. Environmental Management is also important to address environmental factors that contribute to the spread of diseases [47].

Water, Sanitation, and Hygiene (WASH) is important to improve water quality, sanitation, and hygiene practices to reduce the spread of waterborne and foodborne diseases. This along with environmental monitoring is important with the parameters such as water and soil quality for contaminants that may affect human and animal health. There must also be the management of habitats to reduce the risk of disease transmission from wildlife to humans and livestock [5,48].

Implementing effective interventions and control measures within the One Health framework is crucial for addressing the complex health threats that arise at the human-animal-environment interface [48]. Integrated surveillance systems, antimicrobial stewardship, biosecurity measures,

environmental management, and public awareness and community engagement are all vital components of a successful One Health approach. By adopting these strategies, stakeholders can work together to prevent and control diseases, improve public health outcomes, and ensure sustainable health for humans, animals, and the environment [48].

2.26 Antimicrobial Stewardship: Principles and Implementation

Antimicrobial stewardship is a critical component of the One Health approach, aiming to optimize the use of antimicrobials to combat the growing threat of antimicrobial resistance (AMR). This strategy involves coordinated efforts across human, animal, and environmental health sectors to ensure that antimicrobials are used judiciously and effectively [49].

a. **Appropriate Use of Antimicrobials**

Ensuring that antimicrobials are used only when necessary and appropriate helps to preserve their efficacy and reduce the development of resistance. This involves prescribing antimicrobials based on evidence-based guidelines and ensuring they are targeted to the specific pathogens identified [49].

b. Optimal Selection, Dosage, and Duration

The right dosage of antimicrobials and for the right duration minimizes the risk of resistance and adverse effects. This requires clinicians to follow protocols for antimicrobial selection, adjusting treatment based on patient response and pathogen susceptibility[49]. There is need for reducing unnecessary exposure to antimicrobials helps to prevent resistance and preserves the microbiome.

Avoiding the use of antibiotics for viral illnesses and utilizing diagnostic techniques to confirm bacterial infections prior to medication are two strategies[49].

c. Monitoring and Surveillance

Tracking antimicrobial use and resistance patterns helps to identify trends, assess the impact of stewardship interventions, and guide policy decisions. Implementing robust surveillance systems to collect data on antimicrobial prescriptions and resistance in both human and animal health sectors [50]. Educating healthcare providers, veterinarians, and the public about the prudent use of antimicrobials is essential for effective stewardship. Providing continuous education programs and resources to ensure that all stakeholders are informed about the principles and practices of antimicrobial stewardship [50].

2.27 Implementation of Antimicrobial Stewardship in Agriculture

Antimicrobial stewardship in agriculture is vital to address the rising threat of antimicrobial resistance (AMR), which can spread from animals to humans and the environment [51]. Effective stewardship ensures that antimicrobials are used judiciously in livestock, aquaculture, and crop production. There are numerous strategies for implementing antimicrobial stewardship in agriculture. There is the need to establish evidence-based guidelines for antimicrobial use in livestock, aquaculture, and crop production. These guidelines should be specific to species, types of infections, and local resistance patterns. Scientist must ensure that farmers and veterinarians adhere to these guidelines through training and awareness programs. Veterinary Prescription requires that antimicrobials be prescribed by licensed veterinarians based on a diagnosis and

susceptibility testing wherever possible. There is need to encourage regular veterinary consultations to guide appropriate antimicrobial use and health management practices [52].

There is need to implement systems to monitor and record antimicrobial use at the farm level. This data helps to track usage patterns and identify areas for intervention. There is also the need to establish surveillance programs to monitor antimicrobial resistance in animal pathogens. This includes sampling and testing animals, farm environments, and products [52].

2.28 Alternative Approaches to Antibiotics in Agriculture

The increasing concern over antimicrobial resistance (AMR) has prompted the exploration of alternative approaches to antibiotics in agriculture. Probiotics, vaccines, and other alternatives offer promising strategies to promote animal health, improve production efficiency, and reduce the reliance on antimicrobials.

2.28.1 Probiotics

In agriculture, probiotics modulate the gut microbiota, improve nutrient absorption, and strengthen the host immune response [53]. Probiotics have been extensively studied in livestock production to promote growth, prevent diseases, and improve feed efficiency [53]. Common probiotic strains include lactobacilli, bifidobacteria, and bacilli. Probiotics are live microorganisms that provide health benefits when given in adequate amounts[53].

In aquaculture, probiotics are used to maintain water quality, enhance the growth of beneficial microorganisms, and suppress the growth of pathogens. They are often incorporated into feed or

applied directly to the culture environment. Probiotics have demonstrated efficacy in reducing the incidence and severity of gastrointestinal infections, respiratory diseases, and other common ailments in livestock and fish [54]. Studies have shown that probiotics can improve growth rates, feed conversion efficiency, and overall performance parameters in agricultural animals. Selecting the appropriate probiotic strains for specific animal species and health conditions is critical for achieving desired outcomes. It is also important to ensure the viability of probiotic microorganisms throughout the manufacturing process, storage, and administration is essential for efficacy [54].

2.28.2 Vaccines

Biological preparations known as vaccines work by boosting the host's immune system to produce defenses against particular infections. Vaccines are used in agriculture to protect livestock from infectious diseases and lessen the need for antibiotic treatment. Numerous infectious diseases in livestock, such as bacterial, viral, and parasite infections, can be prevented by vaccines. They are delivered via injectable, oral, or intranasal methods to promote immunity [55].

Aquaculture frequently uses vaccination to shield fish and shellfish from bacterial infections, infectious pancreatic necrosis (IPN), and viral hemorrhagic septicemia (VHS). Animal welfare and production efficiency have improved as a result of vaccines' remarkable efficacy in containing outbreaks and lowering the incidence of infectious illnesses in farm animals [55].

By preventing diseases that would otherwise require antibiotic treatment, vaccines contribute to reducing the need for antimicrobials and the risk of AMR. Developing, producing, and administering vaccines can be costly and require infrastructure and expertise. Ensuring affordable access to vaccines for small-scale farmers is a challenge in many regions. The

efficacy of vaccines may vary depending on factors such as antigenic variation, host genetics, and environmental conditions [56].

In order to improve animal health and performance, plant-derived compounds with antimicrobial qualities, such as essential oils, herbs, and spices, are also used as feed additives. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial gut microorganisms, improving immune function and gut health [57].

Viruses that infect and kill specific bacteria called bacteriophages, offer targeted antimicrobial activity against pathogens in livestock and aquaculture. Immune Modulators that modulate the host immune response, such as cytokines, antibodies, and immunomodulatory compounds, which can enhance immune function and reduce susceptibility to infections [58]. The use of alternative approaches to antibiotics in agriculture holds promise for addressing the challenges of antimicrobial resistance while maintaining animal health and productivity. Probiotics, vaccines, and other alternatives offer effective strategies for preventing and controlling infectious diseases, reducing the need for antibiotics, and promoting sustainable agricultural practices. Continued research, investment, and education are essential to optimize the use of these alternatives and ensure their successful integration into agricultural systems globally [58].

2.29 Role of Policies in Controlling Antibiotic Use

The control of antibiotic use is a critical component of efforts to combat antimicrobial resistance (AMR) and safeguard public health. Policies and legislation play a central role in regulating antibiotic use in human medicine, veterinary medicine, and agriculture [59]. Many countries have implemented regulations requiring antibiotics to be prescribed by licensed healthcare professionals, such as physicians or pharmacists, to ensure appropriate use. Hospitals and

healthcare facilities may be required to establish antibiotic stewardship programs to promote judicious antibiotic use, monitor prescribing practices, and prevent overuse and misuse[59].

Veterinarians may be required by law to provide antibiotics to animals only after a diagnosis has been made and only to treat bacterial infections. To make sure that antibiotic residues in meat, milk, and other animal products don't surpass safety limits, regulations frequently outline withdrawal periods for antibiotics used in animals raised for food [60]. Many nations have passed laws limiting the use of antibiotics that are medically significant in agriculture, especially when it comes to growth promotion. To guarantee correct diagnosis and treatment, regulations may mandate that antibiotics used in animal agriculture be administered under a licensed veterinarian's supervision[60].

To combat AMR, numerous nations have created national action plans. In order to fight AMR, Nigeria also implemented a nationwide action plan that was created by the NCDC and ran from 2017 to 2022. In addition to surveillance and monitoring activities, these programs usually incorporate tactics to enhance antibiotic stewardship in veterinary medicine, healthcare settings, and agriculture.

Governments and health organizations issue guidelines and recommendations for antibiotic use in various settings, including clinical practice guidelines for healthcare providers and best management practices for veterinarians and farmers[61].

Policymakers may use incentives, such as subsidies or tax breaks, to encourage compliance with antibiotic stewardship practices. Conversely, penalties may be imposed for non-compliance, such as fines or regulatory sanctions[61]. Studies have shown that regulatory interventions, such as prescription regulations and antibiotic stewardship programs, can lead to reductions in antibiotic

prescribing and consumption in human and veterinary medicine. Policies aimed at controlling antibiotic use have been associated with decreases in the prevalence of antibiotic-resistant bacteria in healthcare settings, animal populations, and the environment [61].

Coordinating antibiotic stewardship efforts across countries and regions is challenging due to differences in regulatory frameworks, healthcare systems, and cultural practices. Ensuring compliance with antibiotic use policies and regulations, particularly in agriculture, can be difficult due to factors such as limited resources, lack of oversight, and resistance from stakeholders[60].

2.30 Policy Development and Implementation

Effective policy development and implementation are essential for combating AMR and preserving the efficacy of antibiotics. Policymakers can take the following steps to address AMR: Implement comprehensive legislation that regulates the use of antibiotics in human medicine, veterinary medicine, and agriculture, incorporating principles of antimicrobial stewardship and One Health[60].

Allocate resources for the establishment and maintenance of robust surveillance systems to monitor antibiotic use, antimicrobial resistance patterns, and healthcare-associated infections, providing data-driven insights for policy decision-making [61]. Support initiatives to promote judicious antibiotic use in healthcare settings, veterinary practices, and food production systems through education, training, and quality improvement programs. Encourage cooperation and coordination between government organizations, medical professionals, veterinarians, researchers, industry participants, and foreign partners in order to create and carry out comprehensive AMR control programs[61].

Incentivize Research and Innovation: Provide funding and incentives for research and innovation in AMR, including the development of new antibiotics, alternative treatments, diagnostics, and vaccines, as well as technologies for AMR surveillance and control. Addressing antimicrobial resistance (AMR) requires collaborative efforts, effective policy development, and international cooperation [62]. The interconnected nature of AMR necessitates collaborative approaches that transcend national boundaries and disciplinary boundaries. International collaborations play a crucial role in pooling resources, facilitating knowledge exchange, promoting harmonization, and enhancing capacity building to address AMR effectively. Policymakers have a key role in creating and carrying out laws that govern the use of antibiotics, encourage antimicrobial stewardship, and support AMR control research and innovation[62].

Healthcare professionals, veterinarians, and policymakers must work together to implement evidence-based interventions and best practices for antimicrobial stewardship. Investments in surveillance, monitoring, and research are critical for monitoring AMR trends, identifying emerging threats, and developing effective control strategies. Collaboration and sharing of information is essential in this fight to address AMR comprehensively and sustainably[63]. By embracing the principles of One Health, promoting international collaborations, and implementing evidence-based policies and interventions, it is possible to mitigate the spread of AMR [63].

Addressing AMR requires a One Health approach which involves integrating human health, with animal health, and environmental sectors in the health measures.. Policies must consider interconnectedness of these domains to effectively control antibiotic use and mitigate AMR. Policies and legislation are essential tools for controlling antibiotic use and combating antimicrobial resistance. Regulatory frameworks, policy instruments, and national action plans

play a crucial role in promoting antibiotic stewardship, reducing antibiotic consumption, and mitigating the spread of AMR [64]. However, challenges remain in ensuring global coordination, enforcement, and compliance with antibiotic use policies, highlighting the need for continued efforts and collaboration across sectors and stakeholders[64].

2.31 Future Directions and Research Gaps in Antimicrobial Resistance

The field of antimicrobial resistance (AMR) continues to evolve, presenting new challenges and opportunities for research. Identifying research gaps is essential for guiding future efforts to combat AMR effectively. There is a need for more comprehensive research that integrates human health, animal health, and environmental factors to better understand the complex dynamics of AMR transmission and dissemination [65]. Understanding the mechanisms and pathways of AMR transmission between humans, animals, and the environment is crucial for developing targeted interventions and control strategies. Research into alternative approaches to antibiotics, such as probiotics, phage therapy, and immunomodulators, is needed to diversify treatment options and reduce reliance on traditional antimicrobials [65].

Effective disease control and management requires stepping up worldwide monitoring efforts to track the transmission of resistant organisms across borders, detect new resistance threats, and track AMR trends. analyzing methods to encourage the appropriate use of antibiotics in food production systems and looking into how the use of antibiotics in agriculture affects AMR in people, animals, and the environment. Evaluating AMR's effects on the economy and society, such as medical expenses, lost productivity, and social inequalities, in order to guide resource allocation and policy choices [66].

2.32 Technological Advances

Recent technological advances have revolutionized our ability to detect and characterize antimicrobial-resistant pathogens, offering new opportunities for AMR research and surveillance. Next-generation sequencing technologies enable rapid and high-resolution genomic analysis of bacterial pathogens, allowing for the identification of resistance genes, mutations, and genetic determinants of AMR [67].

Metagenomic sequencing techniques enable the comprehensive analysis of microbial communities in various environments, providing insights into the diversity and dynamics of antibiotic-resistant bacteria. Rapid diagnostic tests that can accurately detect antibiotic-resistant pathogens at the point of care are essential for guiding clinical decision-making and optimizing antibiotic treatment [68].

Machine learning algorithms and artificial intelligence tools can analyze large-scale genomic and clinical data to predict antibiotic resistance patterns, identify novel resistance mechanisms, and optimize treatment regimens[69]. Nanomaterials and nanodevices show promise for the development of novel antimicrobial agents, drug delivery systems, and diagnostic platforms with enhanced efficacy and specificity. Microfluidic-based systems offer miniaturized and automated platforms for antimicrobial susceptibility testing, microbial culture, and high-throughput screening of antibiotic compounds [69].

The future of AMR research lies in addressing key knowledge gaps, leveraging technological innovations, and adopting holistic approaches to combat this global health threat [70]. We can improve our knowledge of AMR, create efficient interventions, and ensure that antibiotics continue to be effective for future generations by establishing research priorities, embracing technological advancements, and encouraging interdisciplinary collaboration[70]. Antimicrobial resistance (AMR) is a complex and multifaceted problem that transcends national borders and

requires coordinated efforts on a global scale. International collaborations play a crucial role. Collaborative research initiatives allow countries to share resources, expertise, and data, enabling more comprehensive and impactful studies on AMR [70].

International collaborations facilitate the exchange of knowledge, best practices, and lessons learned from different regions and healthcare systems, fostering innovation and accelerating progress in AMR research and control [71]. International cooperation ensure a cohesive strategy to addressing this global health problem by promoting harmonization and consistency in AMR control efforts through the alignment of policies, standards, and surveillance systems across nations. In low- and middle-income nations, collaborative projects help create capacity, enabling researchers, policymakers, and healthcare practitioners to successfully manage antimicrobial resistance (AMR) in their particular contexts [71].

Endnotes

1. O.S Akinsuyi, O.Q. Orababa, O.M. Juwon, I.O. Oladunjoye, E.T. Akande, M.M. Ekpueke & Emmanuel, *One Health approach, a solution to reducing the menace of multidrug-resistant bacteria and zoonoses from domesticated animals in Nigeria–A review*, **Global Biosecurity**, *3,* 2021.

2. A.P Akinduti, O. Ayodele, B.O. Motayo, Y.D. Obafemi, P.O. Isibor & O.W. Aboderin, *Cluster analysis and geospatial mapping of antibiotic resistant Escherichia coli O157 in southwest Nigerian communities*, **One Health,** *15*, 2022. p.100447.

3. O.S. Fasiku, O.O. Omotosho, S.O. Ajulo, E.E. Adesanwo, O.D. Oni, A.A. Awoyele, O.A. Okunlade & V.O. Adetunji, *Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus in Pigs in Ibadan, Nigeria.* **In Medical Sciences Forum** (Vol. 24, No. 1), 2023, pg. 8, MDPI.

4. O.I. Falodun, N.G. Buekor & A. Victor *Multidrug-resistant Enterococcus and coagulase-negative Staphylococcus isolates from poultry farms and nearby wells in Ibadan, Nigeria and their resistant genes*, **One Health Bulletin**, *4*(3), 2024, pg. 117-123.

5. K.O.E. Ada, E.C. Clare, O.E.S. Chidinma, U.O. Goodluck & A. Ei, *Isolation of multidrug resistant and extended spectrum β-Lactamase producing bacteria from faecal samples of piggery farms in Anambra State, Nigeria*, **Am J Infectious Dis Microbiol,** *9*(4), 2021, pp.106-113.

6. O.S. Egbule, B.C. Iweriebor & E.I. Odum, *Beta-Lactamase-producing Escherichia coli isolates recovered from pig handlers in retail shops and Abattoirs in selected localities in Southern Nigeria: Implications for public health,* **Antibiotics,** *10*(1), 2020, pg. 9.

7. J. Muleme, C. Kankya, M. Munyeme, D. Musoke, J.C. Ssempebwa, J.B. Isunju, R. Wambi, B.E. Balugaba, T. Sekulima, R.K. Mugambe, & S. Cadmus, *Phenotypic Characterization and Antibiograms of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolated at the Human-Animal-Environment Interface Using a One Health Approach Among Households in Wakiso District, Uganda,* **Infection and Drug Resistance,** 2023, pp. 2203-2216.

8. D.A. Gado, D.O. Ehizibolo, C.A. Meseko, N.E. Anderson, & P.W. Lurz, *Review of emerging and re-emerging zoonotic pathogens of dogs in Nigeria: Missing link in one health approach*, **Zoonotic Diseases**, *3*(2), 2023, pg. 134-161.

9. N.O. Oloso, S. Fagbo, M. Garbati, S.O. Olonitola, E. J. Awosanya, M.K. Aworh, H. Adamu, I.A. Odetokun, & F.O. Fasina, *Antimicrobial resistance in food animals and the environment in Nigeria: A review,* **International journal of environmental research and public health,** *15*(6), 2018, pg. 1284.

10. O.I. Falodun, & A.A. Ameh. *Multidrug resistance and virulence genes in Campylobacter isolates from poultry waste in Ibadan, Nigeria,* **One Health Bulletin**, *4*(1), 2024, pp. 39-46.

11. I.H. Igbinosa, C.N. Amolo, A. Beshiru, O. Akinnibosun, A.G. Ogofure, M. El-Ashker, M. Gwida, A.I. Okoh & E.O.Igbinosa, *Identification and characterization of MDR virulent Salmonella spp isolated from smallholder poultry production environment in Edo and Delta States, Nigeria,* **PloS One,** *18*(2), 2023, pg. e0281329.

12. O.E. Ojo, A.M. Iledare, E.A. Amosun, & M.A. Dipeolu, *Antimicrobial use and detection of cefotaxime-resistant Enterobacteriaceae in the pig production chain, Ogun State, Nigeria,* **Revue d'élevage et de médecine vétérinaire des pays tropicaux,** *72*(4), 2019, pp.147-154.

13. A.M. Ndahi, I.O. Fagbamila, I. Adebiyi, E. Ekeng, S. Adeleke, V. Adetunji, R.S. Hendriksen, C. Ryan, H. Patel, T. Chisnall, & D. Verner-Jeffreys, 2023, *Establishing a One Health AMR Community of Practice in Nigeria. One Health Cases*, (2023), pg. ohcs20230030.

14. A.O. Ajayi, F.C. Samuel-Osamoka, O.P. Adejoh, & O.A. Popoola, *Antibiotic resistance among bacteria isolated from pigs in selected farms in Ekiti State, Nigeria*, **Dutse Journal of Pure and Applied Sciences,** *9*(4b), 2023, pp. 337-346.

15. O.I. Falodun & O.A. Waleola, *Antibiotic resistance and virulence genes in Campylobacter species from pig and cattle samples in Ibadan, Nigeria,* **European Journal of Biological Research,** *14*(1), 2024, pp. 33-44.

16. L.M. Mamfe, C.A.Akwuobu, & E.O. Ngbede, *Phenotypic detection, antimicrobial susceptibility and virulence profile of staphylococci in the pig production setting, Makurdi, Nigeria,* **Access Microbiology,** *3*(12), 2021, pg. 000293.

17. I.A. Raufu, O.A. Ahmed, A. Aremu, J.A. Ameh, R.E. Timme, R.S. Hendriksen, & A.G. Ambali, *Occurrence, antimicrobial resistance and whole genome sequence analysis of Salmonella serovars from pig farms in Ilorin, North-central Nigeria.* **International Journal of Food Microbiology,** *350*, 2021, pg.109-245.

18. A.V. Kwaghe, D.E. Okomah, D.E. Aworh, E. Awosanya, C.D. Umeokonkwo, E. Yarai, C. Ameh, & J. Kabir, *Magnitude and trends of ruminants, Pigs and poultry diseases in Taraba State, Nigeria, 2013-2017: implications for public health,* **PAMJ-One Health,** *2*(20). 2020.

19. A.L.K. Abia, & S.Y. Essack, *Antimicrobial research and one health in Africa*, **Springer Nature,** 2023.

20. O.J. JOkorie-Kanu, M.U. Anyanwu, E.V. Ezenduka, A.C. Mgbeahuruike, D. Thapaliya, G. Gerbig, E.E. Ugwuijem, C.O. Okorie-Kanu, P. Agbowo, S. Olorunleke, & J.A. Nwanta, *Molecular epidemiology, genetic diversity and antimicrobial resistance of Staphylococcus aureus isolated from chicken and pig carcasses, and carcass handlers*, **Plos one**, *15*(5), 2020, pg. e0232913.

21. N.F. Ekpunobi, S. Adesanoye, O. Orababa, C. Adinnu, C. Okorie, & S. Akinsuyi, *Public health perspective of public abattoirs in Nigeria, challenges and solutions.* **GSC Biological and Pharmaceutical Sciences,** *26*(2), 2024, pg. 115-127.

22. N. Medugu, M.K. Aworh, K. Iregbu, P. Nwajiobi-Princewill, K. Abdulraheem, D.M. Hull, L. Harden, P. Singh, S. Obaro, A. Egwuenu & S. Thakur, *Molecular characterization of multi drug resistant Escherichia coli isolates at a tertiary hospital in Abuja, Nigeria*. **Scientific reports**, *12*(1), 2022, pg.14822.

23. I.A. Raufu, O.A. Ahmed, A. Aremu, J.A. Ameh, R.E. Timme, R.S. Hendriksen, & A. Ambali, *Antimicrobial and genomic characterization of salmonella Nigeria from pigs and poultry in Ilorin, North-Central, Nigeria,* **Journal of infection in developing countries**, *15*(12), 2021, pg. 1899-1909.

24. M.K. Aworh, P. Nilsson, B. Egyir, F.A. Owusu & R.S.Hendriksen, *Rare serovars of non-typhoidal Salmonella enterica isolated from humans, beef cattle and abattoir environments in Nigeri,* **Plos one,** *19*(1), 2024, pg.e0296971.

25. A.M. Tiamiyu, R.C. Okocha, I.A. Adesina, E.M. Okon, I.O. Olatoye, & O.B. Adedeji, *Antimicrobial resistance: A significant public health issue of both human and veterinary concern,* **Open Health,** *5*(1), 2024, pg. 20230046.

26. O. Omotosho, S. Ukatu, M. Okandeji, O. Okunlade, A. Adeola & B. Emikpe, *Antimicrobial Resistance Profile of Aerobic Nasal Microflora of Nigerian Indigenous Pigs,* **Alexandria Journal of Veterinary Sciences,** *76*(1), 2023.

27. N.B. Alhaji, I.A. Odetokun, M.S. Jibrin, M.K. Lawan, & J. Kwaga, *Antibiotic resistance and mitigation using One Health lens in aquaculture of Northern Nigeria*, **Onderstepoort Journal of Veterinary Research,** *91*(2), 2024, pg. 2165.

28. W.A.A. El-Ghany, *Staphylococcus aureus in poultry, with special emphasis on methicillin-resistant strain infection: a comprehensive review from one health perspective,* **International Journal of One Health**, 7(2), 2021, pg. 257-267.

29. K. Fashae & R.S. Hendriksen, *Diversity and antimicrobial susceptibility of Salmonella enterica serovars isolated from pig farms in Ibadan, Nigeria,* **Folia microbiologica**, *59*, 2014, pp.69-77.

30. O. O. Adebowale, F. A. Adeyemo, N. Bankole, M. Olasoju, H. K. Adesokan, O. Fasanmi, O. Adeyemo, O. Awoyomi, O. Kehinde, & Fasina, F. O. *Farmers' perceptions and drivers of antimicrobial use and abuse in commercial pig production, Ogun State, Nigeria.* **International Journal of Environmental Research and Public Health,** *17*(10), (2020), pg. 3579.

31. A.O. Okunlade, A.O. Ogunleye & A.T.P. Ajuwape, *Detection of mecA gene in oxacillin-resistant Staphylococcus aureus isolated from cattle, pigs, abattoir workers in two municipal abattoirs and their antibiotic profiles,* **International Journal of Environmental Studies**, *81*(4), 2024 , pp. 1813-1822.

32. I. Nwankwo, *Epidemiology of Animal and Human Campylobacter Species Infections in Nigeria: A Retrospective Insight and the Need for One Health Approach in the Prevention and Control,* **Veterinary Sciences and Practices,** *18*(1), 2023, pp. 1-11.

33. O.E. Ojo, E.A. Amosun, O.O. Opebiyi, M.A. Oyekunle, M.A. Dipeolu & E.B. Otesile, *Multidrug resistant enterohaemorrhagic Escherichia coli serogroups in the faeces of hunted Wildlife, Abeokuta, Nigeria,* **Veterinaria Italiana,** *58*(2), 2022.

34. D.D. Ibrahim, S.I. Enem, G. Egwu, D. Dantong, K.O. Jolayemi, & M.S. Gaddafi, *An emerging zoonosis: molecular detection of multidrug-methicillin resistant Staphylococcus aureus from butchers' knives, livestock products and contact surfaces,* **Veterinary Research Communications,** *48*(3), 2024, pp. 1697-1705.

35. O.J. Adefioye, E.D. Olaleye, Y.M. Feruke-Bello, R.O. Fasogbon, I.A. Akinwumi, & O.R. Olulowo, *Genotyping of ESBL-Producing E. coli from Food-producing Animals, Animal Food Products and Humans in South-West, Nigeria,* **Ajayi Crowther Journal of Pure and Applied Sciences,** *3*(1), 2024.

36. E.T. Olubisose, A. Ajayi, A.I. Adeleye, & S.I. Smith. *Molecular and phenotypic characterization of efflux pump and biofilm in multi-drug resistant non-typhoidal Salmonella Serovars isolated from food animals and handlers in Lagos Nigeria.* **One Health Outlook**, *3*, 2015, pp. 1-8.

37. C.J. Iwu, *Prevalence and antibiogram of some swine associated Shiga toxin producing Escherichia coli Serogroups and Salmonella species in Nkonkobe Municipality, Eastern Cape Province, South Africa* **(Doctoral dissertation, University of Fort Hare).** 2015.

38. J C. Esumirhewe, A. Cabal-Rosel, F. Allerberger, B. Springer, & W. Ruppitsch, *Genetic characterization of Escherichia coli and Klebsiella spp. from humans and poultry in Nigeria.* **Access Microbiology,** *5*(7), 2023, pp. 000509.

39. K.O. Akinyemi, C.O. Fakorede, J. Linde, U. Methner, G. Wareth, H. Tomaso & H. Neubauer, *Whole genome sequencing of Salmonella enterica serovars isolated from humans, animals, and the environment in Lagos, Nigeria,* **Bmc Microbiology,** *23*(1), 2023, pg. 164.

40. F.E. Adetunji , E.A. Amosun , I.O. Olatoye , & O.E. Ojo . *Prevalence of Extended Spectrum Beta-Lactamases Producing Enterobacteriaceae in Sheep and Goats from Selected Markets in Ibadan, Oyo-State, Nigeria.* **Archives of Basic and Applied Medicine**, *11*(1), 2023. pp.47-52.

41. I.O. Fagbamila , E. Ramon , A.A. Lettini, M. Muhammad , A. Longo, K. Antonello, Aworh, M.K., Kwaga, P.A. Abdu , J.U. Umoh , & J.A. Kabir. *Assessing the mechanisms of multi-drug resistant non-typhoidal Salmonella (NTS) serovars isolated from layer chicken farms in Nigeria.* **Plos one,** *18*(9), 2023. p.e0290754.

42. O.A. Olowe, O.J. Adefioye, T.A. Ajayeoba, J. Schiebel, J. Weinreich, A. Ali, M. Burdukiewicz, S. Rödiger & P. Schierack, *Phylogenetic grouping and biofilm formation of multidrug resistant Escherichia coli isolates from humans, animals and food products in South-West Nigeria,* **Scientific African,** *6*, 2019. Pg. e00158.

43. C.G. Agu, J.F. Nfongeh, E.C.S. Okoye, B.J. Audu, D. Chukwu, K.N. Anueyiagu, M. Mohammad, & C. Aleruchi, *Prevalence and antimicrobial resistance profiles of faecal Escherichia coli isolates from local chickens in Plateau State, Nigeria: Profils de prévalence et de résistance aux antimicrobiens des isolats fécaux d'Escherichia coli provenant de poulets locaux dans l'État du Plateau, Nigeria*, **African Journal of Clinical and Experimental Microbiology,** *25*(3), 2024, pp. 322-332.

44. E.E. Chukwu, V.N. Ibeh & O.F. Davies-Bolorunduro, *Occurrence of Multi-Drug Resistant Listeria species in Faecal Samples of Poultry Chickens in Rural Farms in Lagos State, Nigeria,* **Advances in Infectious Diseases,** *11*(1), 2021, pp. 49-59.

45. K.N. Anueyiagu, C.G. Agu, U. Umar & B.S. Lopes, *Antimicrobial Resistance in Diverse Escherichia coli Pathotypes from Nigeria,* **Antibiotics**, *13*(10), 2024, pg. 922.

46. O.A. Ayodele , A.M. Deji-Agboola , A.O. Faneye , & P.A. Akinduti. *Characterization and antibiotic susceptibility of E. coli O157: H7 in meat and fish sold in Major Ibadan Markets, Nigeria.* **American Journal of Biomedical Sciences,** *12*(2), 2020. pp.99-106.

47. L.I. Uzairue, & O.B. Shittu, *Salmonella enterica Transmission and Antimicrobial Resistance Dynamics across One-Health Sector*. **In Salmonella-Perspectives for Low-Cost Prevention, Control and Treatment**. **IntechOpen,** 2023.

48. O.G. Fasanmi, O.F. Adekunle, O.J. Okuneye, I.O. Akanbi, E.C. Adegor, O.A. Agbato, B.K. Lamidi, O.O. Emikpe, O.T. Owolabi, O.B. Olusegun, & M.O. Oyedepo, *Evaluation of bacterial load and Multi-drug Antibacterial resistant Salmonella and Clostridium isolates from contaminated tools used in butcheries around Ibadan.* **Mathews Journal of Veterinary Science**, *7*(6), 2023, pg. 1-8.

49. O.U. Lawal, O.A. Adekanmbi & O.O. Adelowo, *Occurrence of methicillin-resistant staphylococci in the pig-production chain in Ibadan, Nigeria*, **The Onderstepoort Journal of Veterinary Research,** *88*(1), 2021, pg. 1959.

50. I.A. Raufu, O.A. Ahmed, A. Aremu, J.C. Chen, J.A. Ameh, R.E Timme, R.S. Hendriksen, & A. Ambali, *Whole Genome and Phenotypic Characterization of Novel QnrB19-positive Salmonella Nigeria serovars from Food Animals in Ilorin, North-central, Nigeria,* 2021

51. D. Soma, I.J.O. Bonkoungou, Z. Garba, F.B.J. Diarra, N.S. Somda, M.F.M. Nikiema, E. Bako, E. Sore, S. N. Sawadogo, N. Barro, & K. Haukka, *Extended-Spectrum Beta-Lactamase-Producing and Multidrug-Resistant Escherichia coli and Klebsiella spp. from the Human–Animal–Environment Interface on Cattle Farms in Burkina Faso*, **Microbiology Research**, *15*(4), 2024, pg. 2286-2297.

52. M.K. Aworh, O. Abiodun-Adewusi, N. Mba, B. Helwigh & R.S. Hendriksen, *Prevalence and risk factors for faecal carriage of multidrug resistant Escherichia coli among slaughterhouse workers*, **Scientific Reports,** *11*(1), 2021, pg. 13362.

53. O.T. Jolapamo, & O.O. Osatoyinbo, *Isolation, Identification and Antibiogram of Bacterial Pathogens Gotten from Cow Meat Obtained from Different Market Sources,* **Funksec here,** *5*(2), 2023, pg. 72-79.

54. I.N. Abdullahi, C. Lozano, C. Simón, M. Zarazaga & C.Torres, *Within-host diversity of coagulase-negative staphylococci resistome from healthy pigs and pig farmers, with the detection of cfr-carrying strains and MDR-S. borealis,* **Antibiotics,** *12*(10), 2023, pg. 1505.

55. O.I. Falodun & E.O. Ikusika, *Extended spectrum and metallo-beta-lactamase Pseudomonas species from poultry and piggery waste.* **MicroMedicine,** *7*(2), 2019, pg. 37-45.

56. A.W. Taylor-Robinson & O.O. Omitola. *Emerging and Re-Emerging Bacterial Zoonoses: A Nigerian Perspective on Control, Prevention and Intervention. In Zoonosis of Public Health Interest***, IntechOpen,** 2022.

57. J. N. Dike-Ndudim, R. N. Ugwuegbu, H.C. Amah, C.W. Ndubueze, & E.S. Anikwo. *Identity and Antibiogram of Bacterial Isolates from Owerri Modern Abattoir, Imo State, Nigeria,* **Journal of Advances in Microbiology**, 2021, pg. 43-54.

58. C.A. Agashi, P.E. Ita, E.S. Daudu, G.T. Bankong, A.M. Olaleye, A.U. Obuo, & I.A. Ikpang, *Characterization of Escherichia coli Prevalence and Antibiotic Resistance in Abattoir Wastewaters in Calabar South, Nigeria*, **World News of Natural Sciences,** *59*, 2025, pg. 44-57.

59. U.O. Nwosu, F.A. Ibiam, C.O. Amadi-Ibiam, C.S. Iroha, C.I. Edemekong, I.U. Peter & I.R. Iroha, *Fecal carriage of extended spectrum beta-lactamase and fluoroquinolone resistant gene in non-typhoidal Salmonella enterica isolates from food-producing animals and humans,* **Journal of Drug Delivery & Therapeutics,** *13*(9), 2023.

60. B. Romiluyi, V.O Itaman, B. Odumosu, I.A. Adeleye & S.I. Smith, *Molecular Characterization of Extended Spectrum Beta lactamase Producing Enterobacteriaceae Isolated from Duck Droppings,* **Nigerian Journal of Microbiology,** 2023.

61. A. Bushen, E. Tekalign & M. Abayneh, *Drug-and multidrug-resistance pattern of Enterobacteriaceae isolated from droppings of healthy chickens on a poultry farm in Southwest Ethiopia,* **Infection and drug resistance**, 2021, pg. 2051-2058.

62. T.G Orum, O.O. Ishola, & O. O. Adebowale, *Occurrence and antimicrobial susceptibility patterns of Salmonella species from poultry farms in Ibadan, Nigeria,* **African Journal of Laboratory Medicine,** *11*(1), 2022, pg. 1606.

63. O. Babatunde, F. Mercy & O.O. Adebola, *Water quality and antimicrobial resistant profile of Escherichia coli isolated from dug wells used for domestic purposes,* **International Journal of Public Health,** *11*(1), 2022, pg. 129-137.

64. O.O. Omitola & A.W Taylor-Robinson, *Emerging and re-emerging bacterial zoonoses in Nigeria: current preventive measures and future approaches to intervention*. **Heliyon,** *6*(6) 2020.

65. E.D Ayobola, W. O. Oscar, & E.F. Ejovwokoghene, *Occurrence of plasmid mediated fluoroquinolone resistance genes amongst enteric bacteria isolated from human and animal sources in Delta State, Nigeria,* **AIMS microbiology,** *7*(1), 2021 pg. 75.

66. O.A Ajayi, A.A. Subulade, O.P. Adejoh, & O.A. Popoola, *Investigation of Antibiotic-Resistant Enterococci in Farm Animals and Vegetables: A case study of Selected Towns in Ekiti State, Nigeria*, **Researchers Journal of Science and Technology,** *4*(5), 2024, pp. 85-100.

67. H.K. Adesokan, O.C Obimdike, & V.O Adetunji, *Informal and formal meat marketing in Ibadan, Nigeria: public health implications from microbial assessment*. **PAMJ-One Health**, *5*(15), 2021.

68. O.J. Adefioye, J. Weinreich, S. Rödiger, P. Schierack, & O.A. Olowe, *Phylogenetic characterization and multilocus sequence typing of extended-spectrum beta lactamase-producing Escherichia coli from food-producing animals, beef, and humans in Southwest Nigeria*, **Microbial Drug Resistance**, *27*(1), 2021, pg. 111-120.

69. M. S.,Gaddafi, Y. Yakubu, M. B. Bello, A. A. Bitrus, A. I. Musawa, B. Garba, H. Lawal, M. A. Aliyu, S. A. Barka, & A. J. Emeka, *Occurrence and antibiotic resistance profiles of Methicillin-resistant (MRSA) in layer chickens in Kebbi, Nigeria*, **Folia Veterinaria** 66, no. 2, 2022, pg. 37-45.

70. A. M. Kamweli, P. Nilsson, B. Egyir, F. A. Owusu, & R. S. Hendriksen, *Rare serovars of non-typhoidal Salmonella enterica isolated from humans, beef cattle and abattoir environments in Nigeria,* **Plos one** 19, no. 1, 2024, pg. e0296971.

71. Velazquez-Meza, Maria Elena, Miguel Galarde-López, Berta Carrillo-Quiróz, & Celia Mercedes Alpuche-Aranda, *Antimicrobial resistance: one health approach,* **Veterinary world** 15, no. 3, 2022, pg. 743.

72. Kim, Dae-Wi, & Chang-Jun Cha, *Antibiotic resistome from the One-Health perspective: understanding and controlling antimicrobial resistance transmission*, **Experimental & molecular medicine** 53, no. 3, 2021, pg. 301-309.

73. B. Aslam, M. Khurshid, M.I. Arshad, S, Muzammil, M. Rasool, N. Yasmeen, T. Shah, T.H. Chaudhry, M.H. Rasool, A. Shahid, & X. Xueshan, *Antibiotic resistance: one health one world outlook,* **Frontiers in cellular and infection microbiology** 11, 2021, pg. 771510.

74. Matar, M. Ghassan, Antoine Andremont, & Wael Bazzi, *Combating antimicrobial resistance-a one health approach*. ***Frontiers in Cellular and Infection Microbiology*** 9, 2020, pg. 458.

75. J. Ling, A. Pruden, A. B. Boehm, P. Alvarez, L. Raskin, T. Kohn, & X. Li, *Integrating environmental dimensions of "One Health" to combat antimicrobial resistance: Essential research needs,* **Environmental Science & Technology** 56, no. 21, 2022, pg. 14871-14874.

76. R. A. Hickman, T. Leangapichart, K. Lunha, J. Jiwakanon, S. Angkititrakul, U. Magnusson, M. Sunde & J. D. Järhult, *Exploring the antibiotic resistance burden in livestock, livestock handlers and their non-livestock handling contacts: a one health perspective,* **Frontiers in Microbiology** 12, 2021, pg. 651461.

77. de Mesquita Souza, M. K. Lim, D. F. Marinho do Monte, Patrícia, E. N. Givisiez , L. B. Rodrigues , O. C. de Freitas Neto, Samuel Kariuki, A. B. Júnior, C. J. Br. de Oliveira, &

W. A. Gebreyes, *Antimicrobial resistance in the globalized food chain: A One Health perspective applied to the poultry industry,* **Brazilian Journal of Microbiology,** 2022, pg. 1-22.

78. X. C. Monger, A. Gilbert, L. Saucier & A. T. Vincent, *Antibiotic resistance: From pig to meat,* **Antibiotics** 10, no. 10, 2021, pg. 1209.

79. P. Sunil, P. Shrestha, & B. Adhikari, *Antimicrobial use in food animals and human health: time to implement 'One Health'approach*, **Antimicrobial Resistance & Infection Control** 9, 2020, pg. 1-5.

80. A. Bilal, M. Khurshid, M. I. Arshad, S. Muzammil, M. Rasool, N. Yasmeen & T. Shah Antibiotic resistance: one health one world outlook, **Frontiers in cellular and infection microbiology** 11, 2021, pg. 771510.

Chapter Three

Methodology

3.1 Study Area

Ibadan, the capital city of Oyo State in southwestern Nigeria, is a prominent agricultural town with a significant number of pig farms. These farms vary in size from small-scale backyard

operations to large commercial establishments, reflecting the diverse nature of pig farming in the city. The pig farming industry in Ibadan plays a vital role in the local economy, providing employment and a source of protein for the population.

3.2 Selection Criteria for Study Sites

The selection of study sites for isolating and characterizing multidrug-resistant bacteria from pig farms in Ibadan was based on several criteria to ensure a representation of the farming landscape and to maximize the relevance of the findings to the One Health approach. A total of three pig farms were sampled from the eleven local governments present within the metropolis of Ibadan city. Among the three farms that were sampled, none of them were resident within the same local government.

Inclusion criteria

1. **Farm Sizes:** Large commercial farms with at least a hundred pigs and above.
2. **Different Management Practices:** Farms that make use of both traditional and modern farming practices. Traditional farms may rely on natural feeding methods and local breeds, while modern farms may use formulated feeds, improved breeds, and advanced veterinary care.
3. **Infrastructure and Facilities:** The level of infrastructure varies, with some farms equipped with modern facilities such as automated feeding systems, biosecurity measures, and waste management systems, while others have more basic setups.
4. **Antibiotic Usage**: Farms with documented use of antibiotics, both prophylactic and therapeutic, to assess the impact of antibiotic usage on the development of multidrug-resistant bacteria were selected for this study

5. **Access and Willingness to Participate:** Farms where owners and managers are willing to provide access for sampling and data collection had their pigs recruited into this study. This was to ensure the cooperation and support from farm staff to facilitate smooth and comprehensive sampling processes.

By adhering to these criteria, the study aimed to obtain a diverse and representative sample of pig farms in Ibadan, providing valuable insights into the presence and characteristics of multidrug-resistant bacteria in the region. This approach aligns with the One Health framework, which emphasizes the interconnectedness of human, animal, and environmental health.

3.3 Sample Collection

The samples collected were fecal samples, soil samples, water samples and swab samples of the workers.

a. **Fecal Samples** - Fecal samples were collected directly from the pigs. Fresh fecal droppings were collected from the ground immediately after defecation to minimize contamination

b. **Soil Samples**- soil samples were collected from areas frequently accessed by the pigs, such as feeding and resting areas. Soil samples were collected using sterile scoops or spatulas from the top layer of soil (approximately 2-5 cm depth) in areas where pigs are active

c. **Water Samples**: Water samples were taken from drinking troughs and the water sources and any standing water within the farm premises. Water samples were collected in sterile bottles. If water was stagnant, samples were taken from the middle and bottom layers using a water sampler to capture potential bacterial stratification

d. **Surface Swabs** – Skin swabs were collected from the hands of workers in the farm. Sterile cotton swabs moistened with sterile saline were used to swab palms of the workers. The swabs were then placed in sterile tubes for transport to the laboratory.

3.4 Isolation of Bacteria

To isolate bacteria from the collected samples, a series of laboratory procedures were employed to ensure the accurate identification and characterization of multidrug-resistant bacteria. The following steps outline the laboratory methods used:

3.4.1 Sample Preparation:

a. Fecal Samples: Approximately 1 gram of fecal matter was homogenized in 9 ml of sterile saline or phosphate-buffered saline (PBS) to create a suspension. Serial dilutions were performed to obtain appropriate concentrations for plating [1].

b. **Soil and Water Samples:** These samples were also homogenized and diluted as necessary. Soil samples were suspended in saline or PBS and vortexed to release bacteria into the solution. Water samples were filtered using membrane filtration, and the filters were then placed on agar plates. C. **Surface Swabs:** Swabs were vortexed in sterile saline or PBS to release bacteria from the swab into the solution.

3.4.2 Plating and Incubation

The prepared suspensions were spread onto selective and differential agar plates using sterile techniques to isolate specific types of bacteria. Aseptic techniques were employed throughout the process to prevent contamination. To isolate a diverse range of bacteria, including potential

multidrug-resistant strains, various types of media were used. The selection of media and incubation conditions was tailored to promote the growth of different bacterial species and to identify resistance patterns. Nutrient Agar (NA) was prepared according to manufacturer's instruction and plates were incubated at 37°C for 24-48 hours. MacConkey Agar (MAC) was used to isolate Gram-negative enteric bacteria. It differentiates lactose fermenters (which appear pink) from non-lactose fermenters (which appear colorless). Plates were incubated at 37°C for 24-48 hours[2].

Mannitol Salt Agar (MSA) is a selective and differential medium for isolating Staphylococci. Media was prepared according to manufacturer's instruction and then plates were incubated at 37°C for 24-48 hours. Eosin Methylene Blue Agar (EMB) which is a selective and differential medium was also used to isolate Gram-negative bacteria and differentiating *Escherichia coli* (which produces a characteristic metallic green sheen). The plates were incubated at 37°C for 24-48 hours. Blood Agar was also used to detect hemolytic activity. Plates were incubated at 37°C for 24-48 hours[2].

After incubation, plates were examined for colony morphology, color, and growth patterns. Representative colonies were picked and sub-cultured on fresh media to obtain pure isolates.

Pure isolates were further analyzed through Gram staining, biochemical tests (e.g., catalase, oxidase tests), and molecular methods (e.g., PCR) to confirm their identity and resistance profiles.

This combination of diverse media and specific incubation conditions ensured the effective isolation of a wide range of bacteria, including multidrug-resistant strains, from various sample

types collected from pig farms in Ibadan. The process was designed to maximize the detection and characterization of potential health threats within the One Health framework[3].

3.5 Characterization of Isolates

After the isolation of bacterial strains from the collected samples, a series of characterization techniques were employed to identify the bacterial species, determine their resistance profiles, and understand their potential health implications. The characterization process included morphological, biochemical, and molecular analyses.

3.5.1 Morphological Characterization

Isolated colonies were observed for characteristics such as shape, size, color, texture, and hemolytic activity on blood agar. Detailed notes and photographs were taken to record colony appearances, aiding in preliminary identification. Gram Staining was used to classify bacteria as Gram-positive or Gram-negative based on cell wall composition. Colony morphology was also used to identify isolates

3.5.2 Biochemical Characterization

A battery of biochemical tests were done to identify the isolates. These include the IMVIC battery of tests to identify *Escherichia coli.*

3.5.3 IMViC Battery of Tests

The IMViC battery of tests is a series of biochemical tests used to identify and differentiate among members of the Enterobacteriaceae family based on their metabolic properties.

1. Indole Test

The Indole test determines the ability of an organism to degrade the amino acid tryptophan to indole. The procedure includes inoculating a tryptone broth tube with a pure culture of the test organism. Then isolates are put in a tube and incubated at 37°C for 24-48 hours. After incubation, 5 drops of Kovac's reagent was added to the broth culture. A red layer on the surface of the broth indicates a positive result, while no color change or a yellow layer indicates a negative result[2].

2. Methyl Red Test

The Methyl Red test assesses the ability of the organism to perform mixed acid fermentation and produce stable acid end products. The isolate is inoculated into a glucose-phosphate broth (MR-VP medium) with a pure culture of the test organism and then incubated at 37°C for 48 hours. Following, is the addition of 5 drops of Methyl Red indicator to the culture. A red color indicates a positive result (acidic pH), while a yellow color indicates a negative result (neutral pH) [2].

3. Voges-Proskauer Test

The Voges-Proskauer test detects the presence of acetoin, a neutral end product of glucose metabolism. MR-VP broth used in the Methyl Red test, but after incubation, transfer 1 ml of the culture to a separate test tube and then incubate at 37°C for 24 hours if a fresh culture is used. Add 0.6 ml of alpha-naphthol followed by 0.2 ml of 40% potassium hydroxide to the test tube. Shake the tube gently and let it stand for 15-30 minutes. A pink to red color indicates a positive result, while no color change or a copper color indicates a negative result[3].

4. Citrate Utilization Test

The Citrate Utilization test determines the ability of an organism to use citrate as its sole carbon source. Inoculate a Simmons Citrate Agar slant with a light inoculum of the test organism and then incubate at 37°C for up to 7 days. A color change from green to blue indicates a positive result, while no color change indicates a negative result[3].

Other tests are also carried out to identify *Staphylococcus aureus*

5. Catalase Test

A small amount of bacterial culture was mixed with hydrogen peroxide and the production of bubble indicated a positive result.

6. Oxidase Test

Bacterial colonies were transferred to an oxidase reagent-soaked filter paper. A color change to dark purple within 30 seconds indicated a positive result.

7. Sugar Fermentation Tests

Bacteria were inoculated into media containing the sugar of interest and a pH indicator. Color changes or gas production indicated fermentation.

3.6 Molecular Characterization

Polymerase Chain Reaction (PCR) was used to amplify specific DNA sequences for the identification of bacterial species and detection of resistance genes. DNA was extracted from bacterial isolates using standard protocols. Specific primers targeting species-specific or resistance genes were used in PCR reactions. Amplification products were analyzed by gel electrophoresis. PCR products or whole-genome DNA were sequenced using Sanger sequencing

or next-generation sequencing (NGS) technologies. Sequencing data were analyzed using bioinformatics tools to identify bacterial species and resistance genes. Molecular detection of resistance genes was used to identify specific genes responsible for antibiotic resistance (e.g., mecA for methicillin resistance in Staphylococcus aureus). PCR with primers specific for known resistance genes was performed. Positive PCR products indicated the presence of these genes in the bacterial isolates[4].

3.7 Antibiotic Susceptibility Testing

Disk diffusion (kirby-bauer) method was used to determine the susceptibility of bacterial isolates to various antibiotics and identify multidrug-resistant strains. Bacterial isolates were spread on Mueller-Hinton agar plates. Antibiotic-impregnated disks were placed on the surface, and plates were incubated. Zones of inhibition around the disks were measured to determine susceptibility[4].

3.8 Molecular Characterization

3.8.1. Deoxyribonucleic Acid (DNA) Extraction (Manual Procedure)

Preparation of Reagents

20% SDS (Sodium Dodecyl Sulfate)

Twenty grams of Sodium Dodecyl Sulfate were carefully measured into a conical flask and dissolved in 80 mL of deionized water with gentle stirring, followed by the addition of more deionized water until a total volume of 100 mL of SDS solution was achieved[1].

Enzymatic Lysis Buffer

Tris base (0.605g) was dissolved in approximately 80 mL of distilled water, and the pH was adjusted to 8.0 using HCl. Separately, 0.372 g of EDTA was dissolved in distilled water and adjusted to pH 8.0 with NaOH. Additionally, 0.584 g of NaCl was dissolved in distilled water. These solutions of Tris-HCl, EDTA, and NaCl were then combined. Finally, 100 mg of lysozyme was added to the combined solution and stirred until completely dissolved[5].

Chloroform Isoamyl Alcohol Solution

Chloroform (96 mL) and 4mL of isoamyl alcohol were measured separately. These liquids were then combined in an amber bottle and thoroughly mixed to ensure homogeneity of the solution.

TE (Tris-EDTA) Buffer

The process involved measuring 10mM Tris-HCl and adjusting its pH to 8.0 with HCl. Following this, 1mM EDTA was measured and its pH adjusted to 8.0 using NaOH. The Tris-HCl and EDTA solutions were then combined in a clean container, after which distilled water was added to achieve the final desired volume.

3.8.2 DNA Extraction

Ten milliliters Nutrient Broth culture of the isolates were prepared and incubated for 24 hours at 37^{o}C. 5mLs was aseptically pipetted into clean centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes after which the bacteria cells had collected as a pellet and the bottom of the tubes. The supernatant was discarded by decanting carefully after which a mixture was prepared by adding 2mL of sterile distilled water to the pellet and dispersing the pellet by pipetting up and down with a sterile pipette[5]. To this was added 400 microliters of 10mg/mL lysozyme, which was also thoroughly mixed by pipetting up and down and then incubated at 37°C for 30 minutes.

Next, 800 microliters of 20% SDS solution was added to the culture and incubated at 60°C for another 30 minutes. Solid NaCl was introduced to achieve a concentration of 1M, specifically 0.187008 grams in a 3.2mL volume, and mixed thoroughly by pipetting. An equal volume of Chloroform Isoamyl alcohol was added, and the mixture was centrifuged at 15000rpm for 5 minutes to facilitate protein separation. Following this, absolute ethanol at a concentration of 67% was added. The DNA precipitate was then carefully removed by spooling it around an applicator stick and allowed to dry for approximately a minute to remove excess alcohol. Finally, the DNA was dissolved in 2µL of TE Buffer[5].

3.8.3 Deoxyribonucleic Acid (DNA) Extraction (Kit Procedure)

The procedure began with harvesting a 24-hour-old culture, containing a maximum of 2 x 10^9 cells, in a microcentrifuge tube by centrifuging for 10 minutes at 5000 x g (7500 rpm), after which the supernatant was discarded. The bacterial pellet was then resuspended in 180 µL of enzymatic lysis buffer and incubated at 37°C for 30 minutes. Following this, 25 µL of Proteinase K and 200 µL of Buffer AL (without ethanol) were added and mixed by vortexing, followed by an additional incubation at 56°C for 30 minutes. Subsequently, 200 µL of ethanol (96-100%) was added to the sample and thoroughly mixed by vortexing to ensure a homogeneous mixture. The resulting mixture was pipetted into a spin column placed in a 2 mL collection tube and centrifuged at ≥6000 x g (8000 rpm) for 1 minute[5].

3.8.4 Polymerase Chain Reaction (PCR)

Stock solution of both the forward and reverse primers was made by adding 698µL of T.E buffer to the reverse primer and 644.9µL of the same T.E buffer to the forward primer. A 1:10 dilution of the primers was made with nuclease free water to create a working solution. A 1:10 dilution

of extracted deoxyribonucleic acid (DNA) was also carried out by adding 90µL of nuclease free water to 10µL of DNA in a 1.5mL Eppendorf tube. The mixture was pipetted up and down to achieve a uniform dispersal of the DNA[3].

A 50µL polymerase chain reaction cocktail was prepared by mixing 25 µL of master mix, 1 µL of forward primer, 1 µL of reverse primer, 1 µL of the template (DNA) and 22 µL of nuclease free water.

The primers used to identify the bacterial isolates were universal primers.

Escherichia coli

Target gene: *uidA* (β-glucuronidase gene)

- Forward (uidA-F): 5′-TGGTAATTACCGACGAAAACGGC-3′
- Reverse (uidA-R): 5′-ACGCGTGGTTACAGTCTTGCG-3′
- Amplicon size: ~366 bp.

Staphylococcus aureus

Target gene: *nuc* (thermonuclease gene)

- Forward (nuc-F): 5′-GCGATTGATGGTGATACGGTT-3′
- Reverse (nuc-R): 5′-AGCCAAGCCTTGACGAACTAAAGC-3′
- Amplicon size: ~356 bp

blaTEM-1 (β-lactamase gene)

- Forward (blaTEM-F): 5′-ATCAGCAATAAACCAGC-3′
- Reverse (blaTEM-R): 5′-CCCCGAAGAACGTTTTC-3′
- Amplicon size: ~516 bp

qnrA (quinolone resistance gene)

- Forward (qnrA-F): 5′-ATTTCTCACGCCAGGATTTG-3′
- Reverse (qnrA-R): 5′-GATCGGCAAAGGTTAGGTCA-3′
- Amplicon size: ~516 bp

mecA (methicillin resistance gene)

- Forward (mecA-F): 5′-AAAATCGATGGTAAAGGTTGGC-3′
- Reverse (mecA-R): 5′-AGTTCTGCAGTACCGGATTTGC-3′
- Amplicon size: ~533 bp

Polymerase chain reaction thermocycling conditions were as follows:

The polymerase chain reaction (PCR) process began with both the initial and actual denaturation steps, which were carried out at 94°C for 30 seconds to separate the DNA strands completely. This was followed by the annealing stage, conducted at 54°C for 60 seconds, allowing the primers to bind specifically to their complementary sequences on the DNA template. Next, the extension process took place at 68°C for 2 minutes, during which the DNA polymerase enzyme synthesized new DNA strands by adding nucleotides to the primed sequences. Finally, a final extension step was performed at 68°C for 5 minutes to ensure complete synthesis of all DNA fragments before the reaction was terminated.

The denaturation, annealing and extension occurred repeatedly in 30 cycles. The machine stops the process automatically and keeps it at 4°C. The product of PCR was submitted to a commercial laboratory for determination of product size and sequencing in order to determine the identity of the selected microorganisms by the base sequence of their 16SrRNA gene[4].

3.8.5 DNA Agarose Gel Electrophoresis

PCR products (amplicons) were separated by electrophoresis on a 1% agarose with Tris-acetate-EDTA buffer (TAE). Where 15µl of the PCR product was mixed with 3 µl loading dye and ran at a voltage of 70V for 30min. After running the electrophoresis, the gel was stained with a mixture of crystal violet (0.0025%) and methyl orange dye (0.0005%) in sterile distilled water

for 30 minutes. This was later viewed under white light to confirm the presence and quality of the bacteria DNA[5].

3.9 Disinfectant Susceptibility Testing of Bacterial Isolates

The susceptibility of the bacterial isolates to commonly used farm disinfectants was also assessed using the agar well diffusion method. Fresh overnight cultures of the test isolates were prepared by inoculating each bacterial strain into nutrient broth and incubating at 37°C for 18–24 hours. After incubation, the bacterial suspension was adjusted to match the turbidity of 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) to ensure uniform inoculum density.

Sterile Mueller-Hinton agar plates were used for the assay. Each plate was uniformly seeded with the standardized bacterial suspension using a sterile cotton swab to create a lawn of growth. The plates were allowed to dry for about 10–15 minutes at room temperature. A sterile cork borer, was used to bore five wells (6 mm in diameter) with equal distance from on each agar plate. Each well was then filled with 100 µL of one of the five commercially available farm disinfectants being evaluated. The disinfectants used were selected based on their widespread application in pig farms and included a range of chemical classes (e.g., quaternary ammonium compounds, iodine-based solutions, phenolics, aldehydes, and chlorine-based products). The disinfectants were used at the concentrations recommended by their respective manufacturers.

Following the addition of disinfectants, the plates were allowed to pre-diffuse at room temperature for 30 minutes and then incubated at 37°C for 24 hours under aerobic conditions.

After incubation, the diameters of the zones of inhibition around each well were measured in millimeters using a transparent ruler. The antimicrobial activity of each disinfectant against the isolates was interpreted as follows:

Sensitive (S): ≥ 20 mm

Intermediate (I): 10–19 mm

Resistant (R): < 10 mm

The results were recorded for each isolate–disinfectant combination, and data were tabulated to reflect the effectiveness of each disinfectant against the bacterial pathogens. These findings were useful in assessing the potential of commonly used disinfectants to control microbial contamination in farm environments.

3.10 Method of Analysis

3.10.1 Statistical Methods Used to Analyze Data

In other to analyze the data collected from the isolation and characterization of multidrug-resistant bacteria, statistical methods were employed. Descriptive statistics were used to summarize the basic features of the data and provide simple summaries about the samples and their characteristics.

3.10.2 Database Management

Microsoft Excel was used for initial data entry, cleaning, and basic analysis. Excel's pivot tables and charting tools provided a straightforward way to manage and visualize preliminary data.

Employing these statistical methods and utilizing robust software tools, the study ensured a rigorous and thorough analysis of the data.

Endnotes

1. A.P. Akinduti, O. Ayodele, B.O Motayo, Y.D. Obafemi, P.O. Isibor & O. W. Aboderin, *Cluster Analysis and Geospatial Mapping of Antibiotic Resistant Escherichia coli O157 in southwest Nigerian communities*, **One Health,** *15*, 2022, pg. 100447.

2. O.S. Fasiku, O.O. Omotosho, S.O. Ajulo, E.E. Adesanwo, O.D. Oni, A.A. Awoyele, O.A. Okunlade & V.O. Adetunji, *Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus in Pigs in Ibadan, Nigeria,* **Medical Sciences Forum,** 24(1), 2023, pg. 8.

3. O.I. Falodun, N.G. Buekor & A. Victor, *Multidrug-resistant Enterococcus and coagulase-negative Staphylococcus isolates from poultry farms and nearby wells in Ibadan, Nigeria and their resistant genes*, **One Health Bulletin**, *4*(3), 2024, pg. 117-123.

4. K.O.E. Ada, E.C. Clare, O.E.S. Chidinma, U.O. Goodluck & A. Eli, *Isolation of multidrug resistant and extended spectrum β-Lactamase producing bacteria from faecal samples of piggery farms in Anambra State, Nigeria*, **Am J Infectious Dis Microbiol,** *9*(4), 2021, pg. 106-113.

5. O.S. Egbule, B.C. Iweriebor & E.I. Odum, *Beta-Lactamase-producing Escherichia coli isolates recovered from pig handlers in retail shops and Abattoirs in selected localities in Southern Nigeria: Implications for public health,* **Antibiotics,** *10*(1), 2020, pg. 9.

Chapter Four

Results and Discussion of Findings

4.1 Biochemical Tests

4.1.1 Biochemical Test Results to Identify *Escherichia coli*

A total of eighteen bacterial isolates obtained from pig samples across various locations in Ibadan were subjected to standard biochemical tests and were identified to be *Escherichia coli*. The results showed that all isolates tested positive for both the Indole and Methyl Red tests, while testing negative for the Voges-Proskauer and Citrate tests. This characteristic IMViC pattern (++--) is consistent with the biochemical profile of *E. coli*, supporting their presumptive identification. The detailed results are presented in Table 4.1.1.

4.1.2 Biochemical Test Results for *Staphylococcus aureus*

A total of 24 isolates suspected to be *Staphylococcus aureus* from all the pig samples collected across various locations in Ibadan were subjected to a series of biochemical and carbohydrate fermentation tests for identification. All isolates tested negative for the Indole and Citrate tests, but were positive for both the Methyl Red and Voges-Proskauer tests, indicating their typical metabolic profile. Additionally, the isolates demonstrated the ability to ferment glucose, lactose, sucrose, and maltose, but not arabinose. This carbohydrate fermentation pattern, in combination

with the biochemical test results, is consistent with the known characteristics of *Staphylococcus aureus*. The detailed results are presented in Table 4.1.2.

Table 4.1.1: Biochemical Test Results to Identify *Escherichia coli*

S/N	Isolate Code	Indole test	Methyl red test	Voges proskaeur test	Citrate test
1	INWSPA 1	+	+	-	-
2	EGBSPA1	+	+	-	-
3	OLFC1	+	+	-	-
4	OLFD 1	+	+	-	-
5	INWFA 1	+	+	-	-
6	INWFB 1	+	+	-	-
7	INWFC 1	+	+	-	-
8	INWFE 1	+	+	-	-
9	INWFG 1	+	+	-	-
10	INWFH 1	+	+	-	-
11	INWFAI 1	+	+	-	-
12	EGBFA 1	+	+	-	-
13	EGBFB 1	+	+	-	-
14	EGBFC 1	+	+	-	-
15	EGBFE 1	+	+	-	-
16	EGBFF 1	+	+	-	-
17	OLHA 1	+	+	-	-
18	INWHB 1	+	+	-	-

Source: Author`s Fieldwork, 2025

+: Positive, -: Negative

Table 4.1.2: Biochemical Test Results for *Staphylococcus aureus*

S/N	Isolate Code	Indole test	Methyl red test	Voges proskaeur test	Citrate test	Glucose Fermentation test	Lactose Fermentation test	Sucrose Fermentation test	Maltose Fermentation test	Arabinose
1	OLSPA2	-	+	+	-	+	+	+	+	-
2	INWSPA 2	-	+	+	-	+	+	+	+	-
3	EGBSPB 2	-	+	+	-	+	+	+	+	-
4	OLFA 2	-	+	+	-	+	+		+	-
5	OLFB 2	-	+	+	-	+	+	+	+	-
6	OLFF 2	-	+	+	-	+	+	+	+	-
7	OLFG 2	-	+	+	-	+	+	+	+	-
8	INWFA 2	-	+	+	-	+	+	+	+	-
9	INWFB 2	-	+	+	-	+	+	+	+	-
10	INWFE 2	-	+	+	-	+	+	+	+	-
11	INWFG 2	-	+	+	-	+	+	+	+	-
12	INWFH 1	-	+	+	-	+	+	+	+	-
13	EGBFA 2	-	+	+	-	+	+	+	+	-
14	EGBFB 2	-	+	+	-	+	+	+	+	-
15	EGBFC 2	-	+	+	-	+	+	+	+	-
16	EGBFD 2	-	+	+	-	+	+	+	+	-
17	EGBFG 1	-	+	+	-	+	+	+	+	-
18	OLW2	-	+	+	-	+	+	+	+	-
19	INWW2	-	+	+	-	+	+	+	+	-

Source: Author`s Fieldwork, 2025

Keys: + Positive, - Negative

Table 4.1.2: Biochemical Test Results for *Staphylococcus aureus* (*Cont.)*

S/N	Isolate Code	Indole test	Methyl red test	Voges proskaeur test	Citrate test	Glucose Fermentation test	Lactose Fermentation test	Sucrose Fermentation test	Maltose Fermentation test	Arabinose
20	EGBW 2	-	+	+	-	+	+	+	+	-
21	OLHB 2	-	+	+	-	+	+	+	+	-
22	INWH A 2	-	+	+	-	+	+	+	+	-
23	INWHB 2	-	+	+	-	+	+	+	+	-
24	EGBHB 2	-	+	+	-	+	+	+		-

Source: Author`s Fieldwork, 2025

Keys: + Positive, - Negative

4.1.3 Gram Staining and Cell Morphology

Gram staining was carried out on 42 bacterial isolates obtained from pig samples collected across different locations within Ibadan. The staining results revealed two major morphological groups. A total of 18 isolates were identified as Gram-negative short rods**,** consistent with members of the family *Enterobacteriaceae*, such as *Escherichia coli*. The remaining 24 isolates were Gram-positive cocci**,** a morphological trait typically associated with *Staphylococcus* species. These observations provide an important preliminary step in classifying the bacterial isolates prior to further biochemical and molecular identification. The full results are presented in Table 4.1.3.

4.1.4 Suspected Identity of Isolates Using Eosine Methlyene Blue and Mannitol Salt Agar for *Escherichia coli* and *Staphylococcus aureus* Respectively

To presumptively identify *Escherichia coli* and *Staphylococcus aureus*, bacterial isolates obtained from various pig-related environmental and biological samples were cultured on selective media. Eosin Methylene Blue (EMB) agar was used to isolate and identify *Escherichia coli*, based on its characteristic metallic green sheen colonies. Mannitol Salt Agar (MSA) was employed to isolate *Staphylococcus aureus*, which ferments mannitol and produces yellow colonies due to the resulting acid production. The isolates showing positive colony characteristics for *E. coli* on EMB and *S. aureus* on MSA were recorded according to their

sample types and locations. The results, summarized in Table 4.1.4, indicate the suspected distribution of both organisms across different sample types including soil, faeces, water, and hand swabs collected from pig farm environments within Ibadan.

Table 4.1.3: Gram Staining and Cell Morphology

S/N	Isolate Code	Gram Reaction	Cell morphology
1	EGBSPA1	Negative	Short rods
2	OLFC1	Negative	Short rods
3	OLFD 1	Negative	Short rods
4	INWFA 1	Negative	Short rods
5	INWFB 1	Negative	Short rods
6	INWFC 1	Negative	Short rods
7	INWFE 1	Negative	Short rods
8	INWFG 1	Negative	Short rods
9	INWFH 1	Negative	Short rods
10	INWFAI 1	Negative	Short rods
11	EGBFA 1	Negative	Short rods
12	EGBFB 1	Negative	Short rods
13	EGBFC 1	Negative	Short rods
14	EGBFE 1	Negative	Short rods
15	EGBFF 1	Negative	Short rods
16	OLHA 1	Negative	Short rods
17	INWHB 1	Negative	Short rods
18	INWSPA 1	Negative	Short rods
19	OLSPA2	Positive	Cocci
20	INWSPA 2	Positive	Cocci

Source: Author`s Fieldwork, 2025

Table 4.1.3: Gram Staining and Cell Morphology (*Cont.)*

S/N	Isolate Code	Gram Reaction	Cell morphology
21	OLFA 2	Positive	Cocci
22	OLFB 2	Positive	Cocci
23	OLFF 2	Positive	Cocci
24	OLFG 2	Positive	Cocci
25	INWFA 2	Positive	Cocci
26	INWFB 2	Positive	Cocci
27	INWFE 2	Positive	Cocci
28	INWFG 2	Positive	Cocci
29	INWFH 1	Positive	Cocci
30	EGBFA 2	Positive	Cocci
31	EGBFB 2	Positive	Cocci
32	EGBFC 2	Positive	Cocci
33	EGBFD 2	Positive	Cocci
34	EGBFG 1	Positive	Cocci
35	OLW2	Positive	Cocci
36	INWW2	Positive	Cocci
37	EGBW2	Positive	Cocci
38	OLHB 2	Positive	Cocci
39	INWHA 2	Positive	Cocci
40	INWHB 2	Positive	Cocci
41	EGBSPB2	Positive	Cocci
42	EGBHB 2	Positive	Cocci

Source: Author`s Fieldwork, 2025

Table 4.1.4: Suspected Identity of Isolates using Eosine Methlyene Blue and Mannitol Salt Agar for *Escherichia coli* and *Staphylococcus aureus* Respectively

Sample Type	Sample Code *Escherichia coli*	Sample Code *Staphylococcus aureus*
OLUYOLE SOIL PA	-	OLSPA2
OLUYOLE SOIL PB	-	-
IBADAN NORTH WEST SOIL PA	INWSPA 1	INWSPA 2
IBADAN NORTH WEST SOIL PB	-	-
EGBEDA SOIL PA	EGBSPA1	-
EGBEDA SOIL PB	-	EGBSPB2
OLUYOLE FAECAL A	-	OLFA 2
OLUYOLE FAECAL B	-	OLFB 2
OLUYOLE FAECAL C	OLFC1	-
OLUYOLE FAECAL D	OLFD 1	-
OLUYOLE FAECAL E	-	-
OLUYOLE FAECAL F	-	OLFF 2
OLUYOLE FAECAL G	-	OLFG 2
IBADAN NORTH WEST FAECAL A	INWFA 1	INWFA 2
IBADAN NORTH WEST FAECAL B	INWFB 1	INWFB 2
IBADAN NORTH WEST FAECAL C	INWFC 1	-
IBADAN NORTH WEST FAECAL D	-	-
IBADAN NORTH WEST FAECAL E	INWFE 1	INWFE 2
IBADAN NORTH WEST FAECAL F	-	-
IBADAN NORTH WEST FAECAL G	INWFG 1	INWFG 2
IBADAN NORTH WEST FAECAL H	INWFH 1	INWFH 1
IBADAN NORTH WEST FAECAL I	INWFAI 1	-
EGBDEDA FAECAL A	EGBFA 1	EGBFA 2
EGBDEDA FAECAL B	EGBFB 1	EGBFB 2
EGBDEDA FAECAL C	EGBFC 1	EGBFC 2
EGBDEDA FAECAL D	-	EGBFD 2
EGBDEDA FAECAL E	EGBFE 1	-
EGBDEDA FAECAL F	EGBFF 1	-
EGBDEDA FAECAL G	-	EGBFG 1
OLUYOLE WATER	-	OLW2
IBADAN NORTH WEST WATER	-	INWW2
EGBDEDA WATER	-	EGBW2
OLUYOLE HAND SWAB A	OLHA 1	-
OLUYOLE HAND SWAB B		OLHB 2
IBADAN NORTH WEST HAND SWAB A		INWHA 2
IBADAN NORTH WEST HAND SWAB B	INWHB 1	INWHB 2
EGBDEDA HAND SWAB A	-	-
EGBDEDA HAND SWAB B	-	EGBHB 2

Source: Author`s Fieldwork, 2025

4.1.5 Hemolysis Test for Isolates

Hemolysis testing was conducted on the bacterial isolates to evaluate their ability to lyse red blood cells on blood agar plates, which can indicate pathogenic potential. The hemolytic patterns observed were classified as Alpha (α) hemolysis – partial hemolysis producing a greenish discoloration, Beta (β) hemolysis – complete hemolysis resulting in a clear zone, and Gamma (γ) hemolysis, no hemolysis, showing no change around the colonies. The majority of isolates exhibited gamma hemolysis, indicating non-hemolytic behavior, while a few displayed beta hemolysis, suggestive of higher virulence. Some isolates also showed alpha hemolysis, indicating partial breakdown of red blood cells. These hemolytic reactions provide supplementary evidence for characterizing the isolates' potential pathogenicity. The complete results are summarized in Table 4.1.5.

4.1.6 Antibiotics Susceptibility Test for *Escherichia coli* (mm)

The antibiotic susceptibility profiles of *Escherichia coli* isolates recovered from various pig-associated sources in Ibadan were evaluated using the disc diffusion method. Each isolate was tested against a panel of commonly used antibiotics for Gram-negative bacteria, including β-lactams, fluoroquinolones, aminoglycosides, and sulfonamides. Results showed high resistance to Cefuroxime (CXM), Ampiclox (ACX), Cefotaxime (CTX), and Amoxicillin-Clavulanate (AUG) among many isolates. In contrast, the isolates demonstrated strong sensitivity to Ofloxacin (OFX), Gentamicin (GN), Cefexime (ZEM), Nalidixic Acid (NA), Levofloxacin (LBC), and Ceftriaxone/Sulbactam (CRO). Some isolates showed intermediate resistance to Nitrofurantoin (NF), Imipenem (IMP), and certain cephalosporins. The full susceptibility results, expressed as zone of inhibition diameters (mm) along with interpretations (S = Sensitive, I = Intermediate, R =

Resistant), are presented in Table 4.1.6. The MAR index was calculated for each *Escherichia coli* isolate to assess the level of resistance to multiple antibiotics. An index above 0.2 typically indicates exposure to high-risk environments where antibiotics are frequently used.

4.1.7 Antibiotics Susceptibility Test for *Staphylococcus aureus*

A total of 24 isolates presumptively identified as *Staphylococcus aureus* from various pig-related samples were subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. The isolates were tested against 12 commonly used antibiotics, including β-lactams, fluoroquinolones, macrolides, and cephalosporins. The susceptibility results showed varying degrees of resistance and sensitivity, with most isolates demonstrating high sensitivity to gentamicin (GN), ofloxacin (OFX), levofloxacin (LBC), and ciprofloxacin (CIP), while resistance was frequently observed against cefuroxime (CXM), cefotaxime (CTX), and erythromycin (ERY) in several isolates. Full results are presented in Table 4.1.7. As shown in Table 4.8, MAR indices for the isolates ranged from 0.3 to 0.9, with a significant number (over 80%) exhibiting indices ≥0.6, suggesting the presence of multidrug-resistant *E. coli* strains. These findings underscore the potential health risks associated with antibiotic misuse or overuse in pig farming environments.

Table 4.1.5: Hemolysis Test for Isolates

Isolate Code	Alpha Hemolysis	Beta- Hemolysis	Gamma – Hemolysis
INWSPA 1	-	-	X
EGBSPA1	X	-	-
OLFC1	-	-	X
OLFD 1	X	-	-
INWFA 1	X	-	-
INWFB 1	-	-	X
INWFC 1	-	X	-
INWFE 1	-	-	X
INWFG 1	-	-	X
INWFH 1	-	-	X
INWFAI 1	-	-	X
EGBFA 1	-	-	X
EGBFB 1	-	X	-
EGBFC 1	-	-	X
EGBFE 1	-	-	X
EGBFF 1	-	-	X
OLHA 1	-	X	-
INWHB 1	-	-	X
OLSPA2	-	-	X
INWSPA 2	X	-	-
EGBSPB2	-	-	X
OLFA 2	-	-	X
OLFB 2	-	-	X
OLFF 2	-	-	X
OLFG 2	-	-	X
INWFA 2	-	-	X
INWFB 2	X	-	-
INWFE 2	-	-	X
INWFG 2	-	X	-
INWFH 1	-	-	X
EGBFA 2	-	-	X
EGBFB 2	-	X	-
EGBFC 2	-	-	X
EGBFD 2	-	-	X
EGBFG 1	-	X	-
OLW2	-	-	X
INWW2	-	-	X
EGBW2	-	-	X
OLHB 2	-	-	X
INWHA 2	-	-	X
INWHB 2	-	-	X
INWSPA 2	X	-	-

Source: Author`s Fieldwork, 2025

Keys: - Absent, x Present

Table 4.1.6: Antibiotics Susceptibility Test for *Escherichia coli* (mm)

Isolate/ Antibiotics	CXM	NF	ACX	CTX	AUG	IMP	OFX	GN	ZEM	NA	LBC	CRO	MAR Index
INWFA 1	6	20	6	6	13	10	25	25	25	25	25	15	0.8
OLHA 1	6	15	6	6	6	6	25	25	23	23	25	22	0.8
EGBFB 1	6	20	6	6	6	6	25	15	15	15	25	10	0.7
EGBFG 1	18	20	6	6	10	10	25	25	25	25	25	25	0.6
EGBSPA 1	6	16	6	6	6	6	25	25	20	20	25	17	0.4
OLFC 1	6	25	6	6	6	6	12	25	25	25	25	10	0.7
OLFD 1	6	6	10	6	6	6	25	25	25	25	25	15	0.7
OLSPA 1	6	6	6	6	6	6	25	25	15	15	25	15	0.8
INWFB 1	6	25	6	6	6	6	25	25	20	20	25	17	0.9
INWFC 1	6	25	6	15	15	25	25	25	25	25	25	20	0.6
INWSPA 1	6	25	15	6	15	10	25	25	25	25	25	17	0.3
INWFE 1	6	25	6	10	25	15	25	25	25	25	25	17	0.7
INWFE 1	6	20	10	6	26	6	25	23	20	20	25	18	0.7
EGBFA 1	18	16	6	8	6	8	25	20	25	25	18	21	0.8
EGBFC 1	6	21	8	6	12	12	25	21	25	25	25	12	0.7
EGBFF 1	12	22	6	6	8	14	25	22	19	19	23	20	0.6
INWHB 1	10	20	6	7	18	10	22	6	16	16	18	16	0.7

Gram negative Antibiotic susceptibility testingKey Aug- Amoxicillin Clavulanate – 30ug, CTX- Cefotaxime -25ug, IMP- Imipenem/ Cilastatin – 10/10 ug, OFX- Ofloxacin – 5ug, GN – Gentamicin- 10ug, NA- Nalidixic Acid – 30ug, NF- Nitrofurantoin – 300ug, CXM- Cefuroxime – 30ug, CRO- Cetriaxone Sulbactam – 45ug, ACX – Ampliclox – 10ug, ZEM- Cefexime – 5ug, LBC- Levofloxacin- 5ug

Source: Author`s Fieldwork, 2025

Isolate/ Antibiotics	CXM	OFX	GN	CTX	ERY	CRO	ZEM	AUG	LBC	AZN	CIP	IMP	MAR Index
OLFA 2	15	25	25	6	25	6	6	17	25	25	25	6	0.6
INWFA 2	25	25	25	15	25	10	25	25	25	25	25	25	0.6
OLFG 2	15	25	25	17	25	25	25	25	25	25	25	25	0.5
EGBSPB 2	25	25	25	10	20	15	17	25	25	25	25	25	0.7
OLSPA 2	6	25	25	6	25	15	10	25	25	25	25	20	0.8
1NWFB 2	10	25	25	10	25	15	12	25	25	25	25	10	0.7
EGBFB 2	10	25	23	6	25	13	6	15	25	25	25	10	0.7
EGBFC 2	15	25	25	20	25	6	6	25	18	25	25	15	0.7
OLHB 2	25	25	25	17	25	6	15	25	15	25	25	20	0.9
EGBFG 2	17	24	25	17	23	25	17	25	25	25	25	15	0.6
INWHB 2	20	25	25	17	25	17	17	25	25	25	25	20	0.7
INWHA 2	25	24	25	25	25	20	25	25	25	25	25	25	0.8
EGBHB 2	12	18	20	6	8	13	12	8	6	6	12	8	0.6
EGBW 2	8	8	8	12	7	22	14	19	20	22	24	25	0.7

Table 4.1.7: Antibiotics Susceptibility Test for *Staphylococcus aureus*

CXM – Cefuroxime (30 µg, 2nd generation cephalosporin), **OFX** – Ofloxacin (5 µg, fluoroquinolone), **GN** – Gentamicin (10 µg, aminoglycoside), **CTX** – Cefotaxime (25 µg, 3rd generation cephalosporin), **ERY** – Erythromycin (15 µg, macrolide), **CRO** – Ceftriaxone (30 µg, 3rd generation cephalosporin), **ZEM** – Cefixime (5 µg, 3rd generation cephalosporin, oral), **AUG** – Amoxicillin-Clavulanate (30 µg, β-lactam/β-lactamase inhibitor), **LBC** – Levofloxacin (5 µg, fluoroquinolone), **AZN** – Azithromycin (15 µg, macrolide), **CIP** – Ciprofloxacin (5 µg, fluoroquinolone), **IMP** – Imipenem (10 µg, carbapenem), **MAR Index** – Multiple Antibiotic Resistance Index (proportion of antibiotics to which an isolate is resistant relative to the total tested)

Source: Author`s Fieldwork, 2025

Table 4.1.7: Antibiotics Susceptibility Test for *Staphylococcus aureus (Cont'd)*

Isolate/ Antibiotics	CXM	OFX	GN	CTX	ERY	CRO	ZEM	AUG	LBC	AZN	CIP	IMP	MAR Index
EGBFG 2	14	18	12	18	8	12	15	16	21	20	20	20	0.6
EGBFD 2	6	10	8	14	25	6	6	22	20	11	13	22	0.7
INWW 2	6	6	6	6	8	6	10	6	12	18	11	13	0.8
OLW 2	8	20	15	25	6	6	22	18	12	14	12	25	0.8
EGBFA 2	25	8	8	22	24	17	14	18	10	6	6	20	0.9
INWFH 2	21	24	9	10	15	18	22	24	18	10	6	20	0.7
INWFG 2	22	20	14	16	12	10	6	12	6	12	25	6	0.6
INWFE 2	25	10	12	19	24	18	8	10	17	12	18	21	0.7
OLFF 2	25	14	22	20	6	6	15	18	8	12	14	24	0.7
OLFB 2	20	18	16	25	6	15	10	18	22	8	22	22	0.6

CXM – Cefuroxime (30 µg, 2nd generation cephalosporin), **OFX** – Ofloxacin (5 µg, fluoroquinolone), **GN** – Gentamicin (10 µg, aminoglycoside), **CTX** – Cefotaxime (25 µg, 3rd generation cephalosporin), **ERY** – Erythromycin (15 µg, macrolide), **CRO** – Ceftriaxone (30 µg, 3rd generation cephalosporin), **ZEM** – Cefixime (5 µg, 3rd generation cephalosporin, oral), **AUG** – Amoxicillin-Clavulanate (30 µg, β-lactam/β-lactamase inhibitor), **LBC** – Levofloxacin (5 µg, fluoroquinolone), **AZN** – Azithromycin (15 µg, macrolide), **CIP** – Ciprofloxacin (5 µg, fluoroquinolone), **IMP** – Imipenem (10 µg, carbapenem), **MAR Index** – Multiple Antibiotic Resistance Index (proportion of antibiotics to which an isolate is resistant relative to the total tested)

Source: Author`s Fieldwork, 2025

4.1.8 Effect of Known Farm Disinfectants on Isolates from this Study

Table 4.1.8 presents the susceptibility of *Escherichia coli* and *Staphylococcus aureus* isolates to five commonly used farm disinfectants. The effectiveness of each disinfectant was assessed based on the diameter of the zone of inhibition (in mm), interpreted as Resistant (R), Intermediate (I), or Susceptible (S). Results show variable responses across isolates, with several demonstrating resistance or intermediate susceptibility, especially to Disinfectants 2 and 5. These findings suggest that while some disinfectants retain efficacy, others may be less effective, potentially due to prolonged or improper usage, highlighting the need for regular monitoring of disinfectant performance in farm environments. This is shown on Table 4.1.8.

4.1.9 Prevalence of *E. coli* and *Staphylococcus aureus* in the Study Samples

The prevalence of *Escherichia coli* and *Staphylococcus aureus* across the different sample types and locations was assessed to understand the extent of contamination and potential public health risks associated with each site. A total of 38 samples were collected from soil, fecal matter, water, and hand swabs in Oluyole, Ibadan North West, and Egbeda. Out of the total, *E. coli* was isolated from 19 samples (50%), while *S. aureus* was detected in 25 samples (66%). The highest frequency of *E. coli* was observed in fecal samples, particularly those from Ibadan North West and Egbeda, indicating a high level of fecal contamination in these areas. *S. aureus* was more widely distributed across sample types, including all water and hand swab samples, reflecting its prevalence in both environmental and human-associated sources. The summary of prevalence by sample type and location is presented in Table 4.1.9.

Table 4.1.8: Effect of Known Farm Disinfectants on Isolates from this Study

Isolate Code	Disinfectant 1 (mm/R/S/I)	Disinfectant 2 (mm/R/S/I)	Disinfectant 3 (mm/R/S/I)	Disinfectant 4 (mm/R/S/I)	Disinfectant 5 (mm/R/S/I)
INWSPA 1	25/ S	6 /R	15 / I	25/ S	15 / I
EGBSPA1	23 / S	25/ S	17 / I	25/ S	25/ S
OLFC1	25/ S	17 / I	17 / I	25/ S	25/ S
OLFD 1	25/ S	20 /S	25/ S	25/ S	25/ S
INWFA 1	8 / R	13 / I	12 / I	8 / R	6 /R
INWFB 1	7 / R	22 / S	14 / I	19 / S	20 /S
INWFC 1	8 / R	12 / I	15 / I	16 / I	21 / S
INWFE 1	25/ S	6 /R	6 /R	22 / S	20 /S

Source: Author`s Fieldwork, 2025

Keys: S-Sensitive, I-Intermediate, R-Resistance

Table 4.1.8: Effect of Known Farm Disinfectants on Isolates from this Study

Isolate Code	Disinfectant 1 (mm/R/S/I)	Disinfectant 2 (mm/R/S/I)	Disinfectant 3 (mm/R/S/I)	Disinfectant 4 (mm/R/S/I)	Disinfectant 5 (mm/R/S/I)
INWFG 1	8 / R	6 /R	10 /R	6 /R	12 / I
INWFH 1	6 /R	6 /R	22 /S	18 / I	12 / I
INWFAI 1	24 / S	17 / I	14 / I	18 / I	10 /R
EGBFA 1	15 / I	18 / I	22 / S	24 / S	18 / I
EGBFB 1	12 / I	10 /R	6 /R	12 / I	6 /R
EGBFC 1	24 / S	18 / I	8 / R	10 /R	17 / I
EGBFE 1	8 / R	6 /R	10 /R	6 /R	12 / I
EGBFF 1	6 /R	6 /R	22 /S	18 / I	12 / I
OLHA 1	24 / S	17 / I	14 / I	18 / I	10 /R
INWHB 1	15 / I	18 / I	22 / S	24 / S	18 / I
OLSPA2	12 / I	10 /R	6 /R	12 / I	6 /R
INWSPA 2	24 / S	17 / I	14 / I	18 / I	10 /R
EGBSPB2	15 / I	18 / I	22 / S	24 / S	18 / I
OLFA 2	12 / I	10 /R	6 /R	12 / I	6 /R
OLFB 2	24 / S	18 / I	8 / R	10 /R	17 / I
OLFF 2	8 / R	6 /R	10 /R	6 /R	12 / I
OLFG 2	6 /R	6 /R	22 /S	18 / I	12 / I

Source: Author`s Fieldwork, 2025

Keys: S-Sensitive, I-Intermediate, R-Resistance

Table 4.1.8: Effect of Known Farm Disinfectants on Isolates from this Study (Cont'd)

Isolate Code	Disinfectant 1 (mm/R/S/I)	Disinfectant 2 (mm/R/S/I)	Disinfectant 3 (mm/R/S/I)	Disinfectant 4 (mm/R/S/I)	Disinfectant 5 (mm/R/S/I)
INWFA 2	24 / S	17 / I	14 / I	18 / I	10 /R
INWFB 2	12 / I	10 /R	6 /R	12 / I	6 /R
INWFE 2	24 / S	17 / I	14 / I	18 / I	10 /R
INWFG 2	15 / I	18 / I	22 / S	24 / S	18 / I
INWFH 1	12 / I	10 /R	6 /R	12 / I	6 /R
EGBFA 2	24 / S	18 / I	8 / R	10 /R	17 / I
EGBFB 2	8 / R	6 /R	10 /R	6 /R	12 / I
EGBFC 2	6 /R	6 /R	22 /S	18 / I	12 / I
EGBFD 2	24 / S	17 / I	14 / I	18 / I	10 /R
EGBFG 1	12 / I	10 /R	6 /R	12 / I	6 /R
OLW2	24 / S	17 / I	14 / I	18 / I	10 /R
INWW2	15 / I	18 / I	22 / S	24 / S	18 / I
EGBW2	12 / I	10 /R	6 /R	12 / I	6 /R
OLHB 2	24 / S	18 / I	8 / R	10 /R	17 / I
INWHA 2	8 / R	6 /R	10 /R	6 /R	12 / I
INWHB 2	6 /R	6 /R	22 /S	18 / I	12 / I
EGBHB 2	8 / R	6 /R	10 /R	6 /R	12 / I

Source: Author`s Fieldwork, 2025

Keys: S-Sensitive, I-Intermediate, R-Resistance

Table 4.1.9 Prevalence of *E. coli* and *Staphylococcus aureus* in the Study Samples

Sample Type	Total Samples	*E. coli* Positive	*S. aureus* Positive
Soil (Oluyole)	2	0	1
Soil (Ibadan North West)	2	1	1
Soil (Egbeda)	2	1	1
Fecal (Oluyole)	7	2	5
Fecal (Ibadan North West)	9	7	5
Fecal (Egbeda)	7	6	4
Water (Oluyole)	1	0	1
Water (Ibadan North West)	1	0	1
Water (Egbeda)	1	0	1
Hand Swab (Oluyole)	2	1	1
Hand Swab (Ibadan North West)	2	1	2
Hand Swab (Egbeda)	2	0	1
Total	38	19 (50%)	25 (66%)

Source: Author`s Fieldwork, 2025

4.1.10 Trends Analysis Based on Isolate Frequency

A trend analysis was conducted to identify patterns in the distribution of *Escherichia coli* and *Staphylococcus aureus* across different sample types. The analysis revealed that *E. coli* was the predominant organism in fecal samples, consistent with its origin as a gut bacterium. In contrast, *S. aureus* was more frequently isolated from soil, water, and hand swab samples, suggesting contamination from skin contact or poor hygiene practices. Water samples exclusively yielded *S. aureus*, pointing to potential hygiene-related contamination rather than fecal input. These trends provide insight into the sources and routes of microbial contamination in the sampled environments. A summary of these trends is presented in Table 4.1.10.

4.1.11 Isolates with the Highest Antimicrobial Resistance and Hemolysis

To highlight the most clinically concerning bacterial isolates in this study, isolates exhibiting the highest Multiple Antibiotic Resistance (MAR) indices alongside their hemolysis patterns were identified. High MAR indices (≥ 0.8) indicate exposure to multiple antibiotics and suggest a significant risk for treatment failure. Notably, both *Escherichia coli* and *Staphylococcus aureus* isolates demonstrated strong resistance profiles. Most of these highly resistant isolates exhibited gamma hemolysis, indicating a lack of red blood cell lysis, except *E. coli* isolate OLHA 1, which showed beta hemolysis, complete lysis, implying potential virulence. Table 4.1.11 summarizes these critical isolates, emphasizing their potential public health relevance.

Table 4.1.10 Trends Analysis Based on Isolate Frequency.

Sample Type	Dominant Isolate Type	Remarks
Feces	*E. coli* > *S. aureus*	Gut bacteria common in feces
Soil	*S. aureus* ≥ *E. coli*	Lower counts, surface contamination likely
Water	*S. aureus* only	Indicates hygiene-related contamination
Hand Swabs	*S. aureus* > *E. coli*	Reflects poor personal hygiene and contamination risk

Source: Author`s Fieldwork, 2025

Table 4.1.11 Isolates with the Highest Antimicrobial resistance and Hemolysis

Isolate Code	MAR Index	Hemolysis Type	Suspected Identity
INWFB 1	0.9	Gamma	*Escherichia coli*
EGBFA 2	0.9	Gamma	*Staphylococcus aureus*
OLHB 2	0.9	Gamma	*Staphylococcus aureus*
INWFAI 1	0.8	Gamma	*Escherichia coli*
OLHA 1	0.8	Beta	*Escherichia coli*

Source: Author`s Fieldwork, 2025

4.1.12 Characteristics and Properties of Partial 16S Ribosomal RNA Sequences from the Samples using nBLAST on GenBank

To confirm the identity of multidrug-resistant isolates with significant hemolytic activity, partial 16S ribosomal RNA gene sequencing was carried out. The sequences obtained were analyzed using the NCBI nucleotide BLAST (nBLAST) tool on GenBank. All five sequenced isolates showed high similarity (≥99%) to known bacterial species, thereby validating their preliminary phenotypic identification. *Escherichia coli* and *Staphylococcus aureus* were the dominant species, with sequence alignments showing full query coverage and E-values of 0.0, indicating highly significant matches. These results reinforce the reliability of culture-based methods when complemented with molecular tools and can be seen in Table 4.1.12.

4. 1.13 Identification of Genes

To further investigate the molecular basis of antibiotic resistance in selected multidrug-resistant isolates, PCR amplification was performed for specific resistance genes. Three Extended-Spectrum Beta-Lactamase (ESBL) genes were targeted: *bla-TEM-1***,** *qnrA***,** and *mecA***.** The results revealed that two out of three *E. coli* isolates (66.7%) harbored both the *bla-TEM-1* and *qnrA* genes, suggesting plasmid-mediated resistance to beta-lactams and quinolones as shown in table 4.1.13 below. All three *Staphylococcus aureus* isolates tested (100%) were positive for the *mecA* gene, indicating methicillin resistance. These findings align with the phenotypic resistance patterns and highlight the clinical importance of monitoring resistance gene dissemination as shown in table 4.1.13.

Table 4.1.12: Characteristics and Properties of Partial 16S Ribosomal RNA Sequences from the Samples using nBLAST on GenBank

Sample ID	Length	Identified species	Details	E-value	Alignment score	Highest query coverage (%)
INWFB 1	374 bp	*Escherichia coli*	99.20% similarity using BLAST 2.10.0N+	0.0	>200	100
EGBFA 2	377 bp	*Staphylococcus aureus*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
OLHB 2	380 bp	*Staphylococcus aureus*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
INWFAI 1	375 bp	*Escherichia coli*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100
OLHA 1	375 bp	*Escherichia coli*	99.47% similarity using BLAST 2.10.0N+	0.0	>200	100

Source: Author`s Fieldwork, 2025

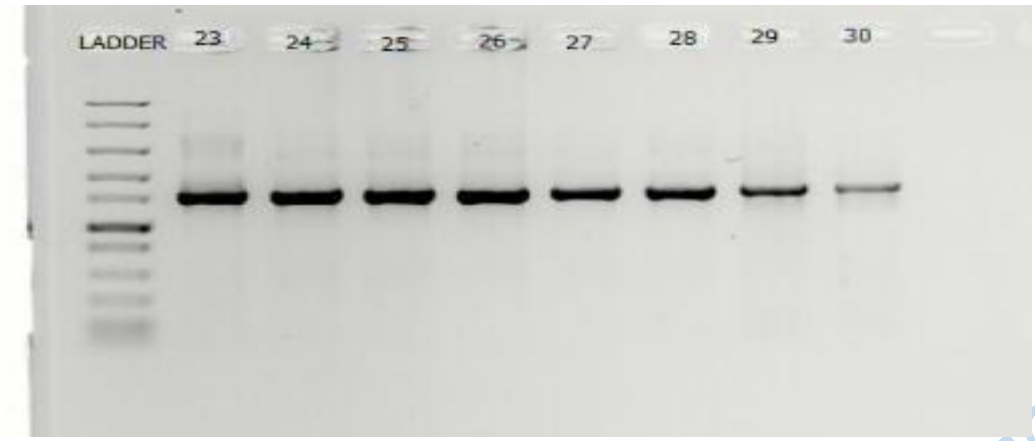


Figure 4.1: Result of the Gel Electrophoresis Done After Genomic

Source: Author`s Fieldwork, 2025

The gel image obtained after running the amplified 16SrRNA gene of the five isolates

Table 4. 1.13: Identification of Genes

ESBL Type	Total Isolate Tested	No of Positives (%)	Isolate Code
bla-Tem – 1	3	2 (66.7)	INWFB 1 ,INWFAI
qnrA	3	3 (100)	INWFB 1 ,INWFAI 1,OLHA 1
mecA	3	2 (66.7)	EGBFA 2, OLHB 2

Source: Author`s Fieldwork, 2025

4.2 Discussion of Findings

This study investigated the isolation and characterization of multidrug-resistant (MDR) bacteria from pig farms in Ibadan, employing a One Health approach to understand the interconnections between animal, human, and environmental health. The findings reveal the presence of MDR bacterial isolates in various samples collected from piggery environments, showing the role of livestock in the emergence and spread of antimicrobial resistance (AMR), transmission pathways, and the urgent need for integrated surveillance and intervention strategies. This study was carried out to isolate and characterize *Staphylococcus aureus* and *Escherichia coli* from collected from pig farms in Ibadan, Nigeria.

The isolates (42) were subjected to biochemical and morphological identification, antimicrobial susceptibility testing, and hemolysis assays, all of which provided insights into their distribution, pathogenicity, and implications for public health under the One Health framework. The analysis of the 38 (water, soil, hand swabs of workers and fecal) samples revealed a relatively high prevalence of both *S. aureus* (66%) and *E. coli* (50%) across various sources. The prevalence trends across sample types revealed fecal samples as the dominant source of both bacteria, particularly from farms in Ibadan North West and Egbeda local Government areas in Oyo State.

In particular, *E. coli* was more frequently isolated from feces, aligning with its biological role as a gut commensal organism that is shed through fecal matter. This observation is consistent with reports of a study which who isolated multiple extended-spectrum β-lactamase (ESBL) producing *E. coli* strains from pig feces in Anambra State [1]. This shows a trend of resistant enteric bacteria in piggery environments. *S. aureus* was more widespread and isolated from a

wider range of samples, including soil, water, hand swabs, and feces. This suggests that *S. aureus* is not only present in the pigs but also thrives in their immediate environment, probably due to poor hygiene practices and biosecurity lapses. This is corroborated by findings from a study who reported a significant occurrence of both methicillin-resistant and methicillin-sensitive *S. aureus* among pigs in Ibadan, showing its zoonotic and environmental transmission potential [2].

S. aureus was detected in all the water samples analyzed, whereas *E. coli* was absent. This suggests that while fecal contamination of water was not always the case, poor hygiene and skin-associated contamination may be contributing to the presence of *S. aureus*. This has serious implications for water safety, especially when the water is used in cleaning, feeding, or comes into contact with humans or animals. Studies have been done that emphasize the importance of environmental hygiene in mitigating *S. aureus* contamination, particularly in carcass handling and meat processing environments, further supporting the findings of this study [11].

Similarly, hand swab samples showed a high prevalence of *S. aureus* and a lower, yet concerning presence of *E. coli*. This further supports the hypothesis of direct and indirect contamination through human handlers, equipment, or surfaces. Poor hand hygiene among farm workers can act as a bridge for bacterial transmission between animals and humans, a phenomenon highlighted in several studies advocating for a One Health approach.

A study reported that 44% of pork samples in Ibadan were contaminated with *Staphylococcus* species, with methicillin-resistant staphylococci (MRS) accounting for 2.5% of the isolates [11]. In a study conducted in Makurdi found that 34.2% of nasal swabs from pigs and handlers were positive for staphylococci, with *S. aureus* constituting 20.8% of these isolates. These findings underscore the widespread nature of *S. aureus* in pig production environments across Nigeria.

For *E. coli*, a study in Anambra State reported a prevalence of 36.4% to 33.5% across different zones. Although our study observed a higher prevalence, the differences could be attributed to variations in farm hygiene practices, sampling methods, and geographical factors [1].

The antimicrobial susceptibility testing in our study revealed high levels of resistance among both *S. aureus* and *E. coli* isolates. Notably, several isolates exhibited multidrug resistance (MDR), with Multiple Antibiotic Resistance (MAR) index values ranging from 0.3 to 0.9. MAR indices above 0.2 typically indicate high-risk sources of contamination where antibiotics are frequently used. Our findings are consistent with those of researchers, who reported a significant occurrence of methicillin-resistant *S. aureus* (MRSA) among pigs in Ibadan, highlighting the potential for zoonotic transmission [5]. Similarly, another study found that 70% of staphylococcal isolates from pigs and handlers in Makurdi expressed methicillin resistance, with 99.2% displaying MDR phenotypes [6].

Regarding *E. coli*, studies have observed that 51.4% of isolates from pig fecal samples in Anambra State were multidrug-resistant, with resistance levels ranging from 7% to 67% against various antibiotics. These patterns mirror our observations and suggest a pervasive issue of antibiotic resistance in pig farming across Nigeria [10].

The findings from this study have revealed a concerning trend in the antimicrobial resistance patterns of *Escherichia coli* and *Staphylococcus aureus* isolates obtained from pig farms in Ibadan, Nigeria. The antibiotic susceptibility profiles showed that both pathogens exhibited extensive resistance to commonly used antimicrobial agents, with several isolates classified as multidrug-resistant (MDR). The presence of MDR strains in this setting signals significant public

health and veterinary concerns, particularly in an environment where routine antibiotic use is widespread and often unregulated.

For *Escherichia coli*, the majority of isolates were resistant to β-lactam antibiotics such as cefuroxime (CXM), amoxicillin-clavulanate (AUG), cefotaxime (CTX), and ampiclox (ACX). Notably, imipenem (IMP) and carbapenem. This aligns with findings from Ada et al. (2021), who reported over 50% resistance to cephalosporins and penicillins in *E. coli* isolates from pig farms in Anambra State. Similarly, studies observed cefotaxime-resistant Enterobacteriaceae in the pig value chain in Ogun State, highlighting the widespread nature of this resistance [8].

Among the *E. coli* isolates in the current study, multiple strains including INWFA 1, INWFB 1, and OLFC1 had multiple antibiotic resistance (MAR) index values ranging from 0.6 to 0.9, indicating resistance to 60 to 90% of the tested antibiotics. MAR index values above 0.2 are considered indicative of bacteria from high-risk environments with significant antibiotic exposure. These values reflect the likelihood that *E. coli* in these pig farms are continuously exposed to antimicrobials either through therapeutic use or as growth promoters.

Likewise, *Staphylococcus aureus* isolates showed high resistance rates to cefotaxime, erythromycin, and azithromycin. While there was moderate susceptibility to gentamicin, ciprofloxacin, and levofloxacin, the widespread resistance to macrolides and β-lactams suggests these antibiotics may no longer be reliably effective in treating staphylococcal infections associated with livestock in this region. *S. aureus* isolates such as OLFA 2, OLFB 2, and EGBFB 2 demonstrated resistance to at least five antibiotics, with MAR indices up to 0.9. These findings are consistent with researchers who reported methicillin-resistant *S. aureus* (MRSA) in pigs in Ibadan and documented significant resistance to multiple antibiotic classes [5].

Similarly, studies found that over 70% of staphylococcal isolates from pigs and handlers in Makurdi were methicillin-resistant, and nearly all were multidrug-resistant. One observation in the current study was the full susceptibility of some *S. aureus* isolates particularly INWHB 2 and INWHA 2 to all tested antibiotics [6]. This may suggest strain variation or limited prior exposure to antimicrobials, and points to the possibility of farm-level differences in antimicrobial practices or hygiene standards.

In addition to antibiotic resistance, the study also evaluated the response of these bacterial isolates to five commonly used farm disinfectants. The results revealed inconsistent efficacy across both *E. coli* and *S. aureus* strains. For *E. coli*, Disinfectants 2, 3, and 5 were largely ineffective, with some isolates showing no zones of inhibition. In contrast, Disinfectants 1 and 4 demonstrated better performance, producing clearer inhibition zones in susceptible strains. However, even these showed limited activity against several MDR isolates. Isolates such as INWFB 1 and OLFC1 were resistant to at least three of the five disinfectants tested.

Similarly, *S. aureus* isolates showed slightly better overall susceptibility to the disinfectants, yet resistance or intermediate reactions were still observed, particularly with Disinfectants 3 and 5. Some isolates, including OLFA 2 and EGBFB 2, were resistant to multiple disinfectants, raising concerns about the effectiveness of existing sanitation protocols on farms. This aligns with previous research which documented limited efficacy of common disinfectants against *S. aureus* in pig production environments [7]. The reduced effectiveness of these agents may be due to resistance mechanisms such as efflux pumps, biofilm formation, or improper usage (e.g., under-dosing or insufficient contact time).

The implication of these findings is that the coexistence of antibiotic-resistant and disinfectant-resistant bacteria in pig farming environments increases the risk of persistent contamination, zoonotic transmission, and reduced treatment options in both veterinary and human medicine. More so, the presence of hemolytic *S. aureus* and *E. coli* strains in the same environment, as shown by the hemolysis test results in this study, suggests the circulation of virulent phenotypes. Beta-hemolytic *S. aureus* strains, which can lyse red blood cells, were particularly prevalent, further indicating a potential for severe infections if these strains are transmitted to humans or susceptible animals.

These findings reveal the need for a multifaceted response rooted in the One Health framework. Firstly, there is a need for routine evaluation and rotation of farm disinfectants based on sensitivity patterns. Disinfectants used on farms must be tested periodically to ensure they are effective against the specific bacterial strains prevalent in each setting. Secondly, antibiotic stewardship programs should be developed and enforced, with regulations to monitor and restrict the use of antimicrobials in livestock. Farmers and farmworkers must also be trained in proper hygiene, antibiotic use, and disinfection practices to minimize the development and spread of resistance.

Furthermore, future research should explore the molecular mechanisms underpinning resistance in these isolates, particularly the presence of resistance genes such as *bla*, *mecA*, and *qac*, which are associated with antibiotic and disinfectant resistance. Such studies will provide a clearer picture of the threat landscape and guide more targeted interventions. This study highlights the dual challenge of antimicrobial and disinfectant resistance among bacterial pathogens isolated from pig farms in Ibadan. The detection of MDR and virulent strains of *E. coli* and *S. aureus*

suggests a pressing need for comprehensive surveillance, biosecurity improvement, and coordinated action across the animal, human, and environmental health sectors. Only through integrated One Health strategies can we hope to curb the growing menace of antimicrobial resistance in agricultural settings.

The hemolysis assays conducted in our study revealed that many *E. coli* isolates exhibited gamma (γ)-hemolysis, indicating non-hemolytic behavior. However, some isolates demonstrated alpha (α) and beta (β)-hemolysis, suggesting the presence of potentially pathogenic variants. For *S. aureus*, a number of isolates showed beta-hemolysis, which indicates virulence associated with the bacteria's ability to lyse red blood cells and cause tissue damage. These findings are in line with those who reported that all coagulase-positive staphylococci from pigs and handlers in Makurdi exhibited resistance to serum bactericidal activity, and 11.3% produced biofilms, enhancing their virulence potential [9].

The high prevalence of MDR *S. aureus* and *E. coli* in pig farms poses significant public health risks. The potential for these pathogens to enter the human population through direct contact, environmental exposure, or consumption of contaminated pork products necessitates a comprehensive One Health approach to mitigate the spread of antimicrobial resistance. A systematic review that highlighted that MRSA prevalence in pigs across Nigeria ranged from 0% to 53.9%, with livestock farm workers exhibiting prevalence rates between 3.1% and 71.4% [10]. These statistics emphasize the interconnectedness of animal and human health and the importance of coordinated efforts to address antimicrobial resistance [15].

The implications of these findings are profound when viewed through the lens of the One Health framework. The isolation of MDR *E. coli* and *S. aureus* from diverse environmental and

biological sources demonstrates the interconnectedness of human, animal, and environmental health. These results resonate with the review which called for an integrated One Health approach to address the challenges of antimicrobial resistance and zoonotic disease transmission in Nigeria [4].

Additionally, the findings of a study done in Uganda emphasize that interventions at the household, farm, and environmental levels are necessary to disrupt the transmission cycle of resistant bacteria [7]. The presence of antimicrobial genes in the isolates was also sough and among the three isolates tested for the *bla-TEM* gene, two (66.7%) were positive, specifically INWFB 1 and INWFAI 1, while OLHA 1 showed no amplification for this gene. Similarly, the *qnrA* gene was detected in two out of three isolates (66.7%), including INWFB 1 and INWFAI 1, suggesting resistance to quinolone antibiotics.

In contrast, all three isolates (100%) tested for the *mecA* gene, namely EGBFA 2, OLHB 2, and one unspecified isolate, were positive, indicating a high likelihood of methicillin resistance. These findings underscore the widespread presence of multiple antibiotic resistance genes among the poultry feed-associated bacterial isolates, raising serious public and veterinary health concerns. The current study contributes valuable data from Ibadan, Nigeria, demonstrating that pig farms can serve as reservoirs for both virulent and drug-resistant bacteria capable of infecting humans and animals alike.

Endnotes

1. K.O.E.Ada, E.C. Clare, O.E.S. Chidinma, U.O. Goodluck & A. Ei, *Isolation of multidrug resistant and extended spectrum β-Lactamase producing bacteria from faecal samples of piggery farms in Anambra State, Nigeria*, **Am J Infectious Dis Microbiol,** *9*(4), 2021, pg. 106-113.

2. O. O. Adebowale, F. A. Adeyemo, N. Bankole, M. Olasoju, H. K. Adesokan, O. Fasanmi, A. Olajoju, K. Olugbenga, & F. O. Fasina, *Farmers' perceptions and drivers of antimicrobial use and abuse in commercial pig production, Ogun State, Nigeria,* **International Journal of Environmental Research and Public Health,** *17*(10), 2020, pg. 3579.

3. A.O. Ajayi, F.C. Samuel-Osamoka, O.P. Adejoh, & O.A. Popoola, Antibiotic resistance among bacteria isolated from pigs in selected farms in Ekiti State, Nigeria, **Dutse Journal of Pure and Applied Sciences,** *9*(4b), 2023, pg. 337-346.

4. O.S Akinsuyi, O.Q. Orababa, O.M. Juwon, I.O. Oladunjoye, E.T Akande, M.M. Ekpueke & A. Emmanuel, *One Health approach, a solution to reducing the menace of multidrug-resistant bacteria and zoonoses from domesticated animals in Nigeria–A review*, **Global Biosecurity**, *3,* 2021.

5. O.S. Fasiku, O.O. Omotosho, S.O. Ajulo, E.E. Adesanwo, O.D. Oni, A.A. Awoyele, O.A. Okunlade & V.O Adetunji,. November, *Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus in Pigs in Ibadan, Nigeria.* **In Medical Sciences Forum** (Vol. 24, No. 1,). 2023, pg. 8

6. L.M. Mamfe, C.A. Akwuobu, & E.O. Ngbede, *Phenotypic detection, antimicrobial susceptibility and virulence profile of staphylococci in the pig production setting, Makurdi, Nigeria.* **Access Microbiology,** *3*(12), 2021, pg. 000293.

7. J. Muleme, C. Kankya, M. Munyeme, D. Musoke, J.C. Ssempebwa, R. Isunju, J.B. Wambi, B.E. Balugaba, T. Sekulima, R.K. Mugambe & S.Cadmus, *Phenotypic Characterization and Antibiograms of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolated at the Human-Animal-Environment Interface Using a One*

Health Approach Among Households in Wakiso District, Uganda. **Infection and Drug Resistance,** 2023, pg. 2203-2216.

8. O.E. Ojo, E.A Amosun, O.O. Opebiyi, M.A. Oyekunle, M.A. Dipeolu & E.B. Otesile. *Multidrug resistant enterohaemorrhagic Escherichia coli serogroups in the faeces of hunted Wildlife, Abeokuta, Nigeria.* **Veterinaria Italiana,** *58*(2), 2022.

9. O.J. Okorie-Kanu, M.U. Anyanwu, E.V. Ezenduka, A.C. Mgbeahuruike, D. Thapaliya, G. Gerbig, E.E. Ugwuijem, C.O. Okorie-Kanu, P. Agbowo, S. Olorunleke & J.A. Nwanta, *Molecular epidemiology, genetic diversity and antimicrobial resistance of Staphylococcus aureus isolated from chicken and pig carcasses, and carcass handlers*, **Plos one**, *15*(5), 202,. pg. e0232913.

10. I.A. Raufu, O.A. Ahmed, A. Aremu, J.C. Chen, J.A. Ameh, R.E. Timme, R.S. Hendriksen, & A. Ambali, *Whole Genome and Phenotypic Characterization of Novel QnrB19- positive Salmonella Nigeria serovars from Food Animals in Ilorin, North-central, Nigeria,* 2021.

11. I.A. Raufu, O.A. Ahmed, A. Aremu, J.C. Chen, J.A. Ameh, R.E. Timme, R.S. Hendriksen, & A. Ambali, *Antimicrobial and genomic characterization of salmonella Nigeria from pigs and poultry in Ilorin, North-Central, Nigeria,* **Journal of infection in developing countries**, *15*(12), 2021, pg. 1899 -1909.

Chapter Five

Conclusion

5.1 Summary of Findings

This study examined the prevalence, resistance patterns, and potential transmission pathways of multidrug-resistant (MDR) bacteria from pig farms in Ibadan, Oyo State, within the framework of the One Health approach. A total of 42 bacterial isolates were recovered from 38 samples comprising fecal matter, soil, water, and workers' hand swabs across three pig farms. Among the isolates, *Staphylococcus aureus* (66%) and *Escherichia coli* (50%) were the most prevalent species. Fecal samples constituted the dominant source of these bacteria, particularly from farms located in Ibadan North West and Egbeda Local Government Areas, underscoring the role of animal waste as a key reservoir of resistant microorganisms.

Antimicrobial susceptibility testing revealed high levels of resistance in both *S. aureus* and *E. coli*, with several isolates showing resistance to multiple classes of antibiotics. The Multiple Antibiotic Resistance (MAR) index of the isolates ranged from 0.3 to 0.9, values well above the 0.2 threshold that signals high-risk sources where antibiotics are frequently or indiscriminately used. These findings strongly indicate that the pig farms investigated are critical hotspots for the dissemination of resistant bacteria.

Further molecular characterization identified the presence of resistance genes such as *bla*, *mecA*, and *qac*, which are linked to β-lactam resistance, methicillin resistance, and antiseptic/disinfectant resistance, respectively. The coexistence of these genetic determinants across isolates highlights the potential for horizontal gene transfer and persistence of resistance traits in both Gram-negative and Gram-positive bacteria.

Overall, the findings demonstrate that pig farms in Ibadan harbor significant reservoirs of MDR bacteria with potential implications for public health, animal health, and environmental safety. The co-occurrence of *E. coli* and *S. aureus* in animal, environmental, and human-related samples illustrates the interconnectedness of resistance transmission pathways, reinforcing the importance of addressing antimicrobial resistance through integrated surveillance, improved biosecurity practices, and prudent antibiotic stewardship under the One Health framework.

5.2 Conclusion

The findings of this study show the rise of antibiotic-resistant bacteria in both animal and environmental samples. The observed resistance patterns, particularly in Gram-negative and Gram-positive bacteria such as *Escherichia coli* and *Staphylococcus aureus*, have implications for public health, animal welfare, and environmental sustainability. The levels of resistance to commonly used antibiotics, including amoxicillin, tetracycline, and cephalosporins, indicate that the current antibiotic use practices in agriculture, healthcare, and the environment are contributing to the development and spread of resistant bacterial strains.

Resistance in these bacteria make effective treatment of infections in humans and animals difficult but also poses environmental risks by contaminating water and soil with resistant

pathogens and resistance genes. These findings align with other global studies that have highlighted the growing challenge of antimicrobial resistance (AMR) in agricultural and environmental settings, further emphasizing the need for a coordinated, multisectoral approach to combat AMR.

The One Health framework, which links human, animal, and environmental health, offers an integrated approach to addressing antibiotic resistance. The study demonstrates the interconnectedness of these health domains and the necessity for collaborative efforts across sectors to effectively mitigate the spread of resistance. However, urgent action is required in policy implementation, regulation, and behavioral changes in both healthcare and agricultural practices.

5.3 Recommendations

1. **Implementation of Strengthened Antimicrobial Stewardship Programs:** The adoption of robust antimicrobial stewardship programs in both human healthcare and veterinary sectors is critical. These programs should focus on reducing unnecessary antibiotic use and ensuring that antibiotics are only prescribed when absolutely necessary, in appropriate doses, and for the recommended duration.
2. **Regulating Antibiotic Use in Agriculture:** Governments and regulatory bodies should enforce stricter regulations on the use of antibiotics in agriculture. Antibiotics should be restricted to therapeutic uses only and not as growth promoters or prophylactic treatments. This would reduce the selective pressure for the development of resistant bacteria in animals.

3. **Environmental Surveillance and Waste Management:** Enhanced environmental surveillance programs should be established to monitor antibiotic resistance in soil, water, and agricultural runoff. These programs should aim to detect resistant pathogens early and track their spread across ecosystems.
4. **Promoting Research and Development:** There is an urgent need to invest in research and development (R&D) to discover new antibiotics, alternative therapies, and diagnostic tools. The development of new classes of antibiotics, as well as non-antibiotic treatments like bacteriophages or antimicrobial peptides, could provide valuable alternatives to combat resistant infections.
5. **Improved Surveillance Systems and Data Sharing:** Integrated surveillance systems that connect human health, animal health, and environmental monitoring should be established. These systems should enable real-time data sharing and collaborative efforts across sectors to track the emergence and spread of resistant bacteria.
6. **Public Awareness and Education Campaigns:** Public awareness campaigns should aim to educate individuals on the importance of completing antibiotic courses, the risks of self-medication, and the need to prevent infections through good hygiene practices and vaccination.
7. **Strengthening Policies and Regulatory Frameworks:** Policymakers should prioritize the implementation and enforcement of stringent regulations on antibiotic use across healthcare, agriculture, and environmental sectors. This includes banning the over-the-counter sale of antibiotics, imposing stricter regulations on the use of antibiotics in animal farming, and ensuring proper monitoring of antibiotic residues in food products and environmental samples.

5.4 Contribution to Knowledge

This study makes the following contributions to knowledge:

1. **Evidence of MDR Bacteria in Pig Farms:** It provides empirical data on the prevalence of multidrug-resistant (*Staphylococcus aureus* and *Escherichia coli*) in pig farms in Ibadan, an area that has received limited research attention compared to poultry and cattle production. This highlights pig farms as critical but underexplored reservoirs of antimicrobial resistance in Nigeria.
2. **High MAR Index Documentation:** The study establishes the occurrence of high Multiple Antibiotic Resistance (MAR) indices, ranging from 0.3 to 0.9, in bacterial isolates from pig farms. This serves as a benchmark for future antimicrobial resistance surveillance in livestock production systems in Nigeria and other low- and middle-income countries.
3. **Molecular Insights into Resistance Genes:** The detection of resistance genes such as *bla*, *mecA*, and *qac* in both Gram-positive and Gram-negative bacteria contributes novel insights into the genetic determinants of resistance in pig farming environments. This finding underscores the potential for gene transfer and persistence of resistance traits across bacterial species.
4. **One Health Methodological Contribution:** By applying the One Health approach to analyze samples from animals, the environment, and farm workers, the study demonstrates a holistic framework for understanding antimicrobial resistance transmission pathways. This interdisciplinary approach can guide integrated surveillance and inform policy interventions in Nigeria and beyond.

5.5 Suggested Areas of Further Research

This study opens up several important areas for future investigation:

1. **Expanded Geographical and Sample Coverage:** Future studies should include a larger number of pig farms across different regions of Nigeria to provide a broader understanding of antimicrobial resistance (AMR) trends in pig farming nationwide.
2. **Longitudinal Surveillance Studies:** Research should focus on monitoring resistance patterns over time to evaluate the impact of interventions such as antibiotic stewardship programs, farm-level biosecurity measures, and stricter regulatory controls on antibiotic use.
3. **Advanced Molecular and Genomic Investigations:** Further studies should explore a wider range of resistance genes and mobile genetic elements, including plasmids and transposons. Whole genome sequencing and other genomic tools could provide deeper insights into resistance mechanisms and gene transfer dynamics.
4. **Occupational and Public Health Risk Assessments:** Future research should assess the risks of resistant bacteria spreading from pig farms to farm workers, surrounding communities, and the environment. Integrated One Health studies combining environmental monitoring, epidemiological surveys, and risk modeling would be particularly valuable.

Bibliography

Journals

Abdullahi I.N., Lozano C., Simón C., Zarazaga M. & Torres C., *Within-host diversity of coagulase-negative staphylococci resistome from healthy pigs and pig farmers, with the detection of cfr-carrying strains and MDR-S. borealis,* **Antibiotics,** *12*(10), 2023, pg. 1505.

Abia A.L.K., & Essack S.Y., eds.,. *Antimicrobial research and one health in Africa*, **Springer Nature,** 2023.

Ada K.O.E., Clare E.C., Chidinma O.E.S., Goodluck U.O. & Ei, A, *Isolation of multidrug resistant and extended spectrum β-Lactamase producing bacteria from faecal samples of piggery farms in Anambra State, Nigeria*, **Am J Infectious Dis Microbiol,** *9*(4), 2021, pg. 106-113.

Adebowale O., Makanjuola, M., Bankole N., Olasoju M., Alamu, A., Kperegbeyi, A., Oladejo, E., Fasanmi O., Adeyemo O. & Fasina F.O., *Multi-drug resistant Escherichia coli, biosecurity and anti-microbial use in live bird markets, Abeokuta, Nigeria*, **Antibiotics**, *11*(2), 2022, pg. 253.

Adebowale, O. O., Adeyemo, F. A., Bankole, N., Olasoju, M., Adesokan, H. K., Fasanmi, O., Olajoju A., Olugbenga K. & Fasina, F. O., *Farmers' perceptions and drivers of antimicrobial use and abuse in commercial pig production, Ogun State, Nigeria,* **International Journal of Environmental Research and Public Health,** *17*(10), 2020, pg. 3579.

Adefioye O.J., Weinreich, J., Rödiger S., Schierack P. & Olowe O.A, *Phylogenetic characterization and multilocus sequence typing of extended-spectrum beta lactamase-producing Escherichia coli from food-producing animals, beef, and humans in Southwest Nigeria*, **Microbial Drug Resistance**, *27*(1), 2021, pg. 111-120.

Adefioye O.J., Olaleye E.D., Feruke-Bello Y.M., Fasogbon R.O., Akinwumi I.A. & Olulowo O.R., *Genotyping of ESBL-Producing E. coli from Food-producing Animals, Animal Food Products and Humans in South-West, Nigeria,* **Ajayi Crowther Journal of Pure and Applied Sciences,** *3*(1), 2024.

Adesokan H.K., Obimdike O.C. & Adetunji V.O., *Informal and formal meat marketing in Ibadan, Nigeria: public health implications from microbial assessment*, **PAMJ-One Health**, *5*(15), 2021.

Adetunji F.E., Amosun E.A., Olatoye I.O. & Ojo O.E., *Prevalence of Extended Spectrum Beta-Lactamases Producing Enterobacteriaceae in Sheep and Goats from Selected Markets in Ibadan, Oyo-State, Nigeria,* **Archives of Basic and Applied Medicine**, *11*(1), 2023, pg. 47-52.

Agashi C.A., Ita P.E, Daudu E.S., Bankong G.T., Olaleye A.M., Obuo A.U. & Ikpang I.A., *Characterization of Escherichia coli Prevalence and Antibiotic Resistance in Abattoir Wastewaters in Calabar South, Nigeria*, **World News of Natural Sciences,** *59*, 2025, pg. 44-57.

Agu C.G., Nfongeh J.F., Okoye E.C.S., Audu B.J., Chukwu D., Anueyiagu K.N., Mohammad, M. & Aleruchi C., *Prevalence and antimicrobial resistance profiles of faecal Escherichia coli isolates from local chickens in Plateau State, Nigeria: Profils de prévalence et de résistance aux antimicrobiens des isolats fécaux d'Escherichia coli provenant de poulets locaux dans l'État du Plateau, Nigeria*, **African Journal of Clinical and Experimental Microbiology,** *25*(3), 2024, pg. 322-332.

Ajayi A.O., Samuel-Osamoka F.C., Adejoh O.P., & Popoola. O.A., *Antibiotic resistance among bacteria isolated from pigs in selected farms in Ekiti State, Nigeria*, **Dutse Journal of Pure and Applied Sciences,** *9*(4b), 2023, pg. 337-346.

Ajayi O.A, Subulade A.A., Adejoh O.P. & Popoola O.A., *Investigation of Antibiotic-Resistant Enterococci in Farm Animals and Vegetables: A case study of Selected Towns in Ekiti State, Nigeria*, **Researchers Journal of Science and Technology,** *4*(5), 2024, pp. 85-100.

Akinduti A.P, Ayodele O., Motayo B.O, Obafemi Y.D., Isibor P.O. & Aboderin O.W., *Cluster analysis and geospatial mapping of antibiotic resistant Escherichia coli O157 in southwest Nigerian communities*, **One Health,** *15*, 2022, pg. 100447.

Akinsuyi O.S, Orababa O.Q., Juwon O.M., Oladunjoye I.O, Akande E.T, Ekpueke M.M. & Emmanuel A., *One Health approach, a solution to reducing the menace of multidrug-resistant bacteria and zoonoses from domesticated animals in Nigeria–A review*, **Global Biosecurity**, *3,* 2021.

Akinyemi K.O., Fakorede C.O., Linde J., Methner U., Wareth G., Tomaso H. & Neubauer H., *Whole genome sequencing of Salmonella enterica serovars isolated from humans, animals, and the environment in Lagos, Nigeria,* **Bmc Microbiology,** *23*(1), 2023, pg. 164.

Alhaji N.B., Odetokun I.A., Jibrin M.S., Lawan M.K. & Kwaga J., *Antibiotic resistance and mitigation using One Health lens in aquaculture of Northern Nigeria*, **Onderstepoort Journal of Veterinary Research,** *91*(2), 2024, pg. 2165.

Anueyiagu K.N., Agu C.G., Umar U. & Lopes B.S., *Antimicrobial Resistance in Diverse Escherichia coli Pathotypes from Nigeria,* **Antibiotics**, *13*(10), 2024, pg. 922.

Aworh M.K., Nilsson P., Egyir B., Owusu F.A. & Hendriksen R.S., *Rare serovars of non-typhoidal Salmonella enterica isolated from humans, beef cattle and abattoir environments in Nigeria*, **Plos one,** *19*(1), 2024, pg. e0296971.

Aworh M.K., Abiodun-Adewusi O., Mba N., Helwigh B. & Hendriksen R.S., *Prevalence and risk factors for faecal carriage of multidrug resistant Escherichia coli among slaughterhouse workers*. **Scientific Reports,** *11*(1), 2021, pg. 13362.

Ayobola E.D., Oscar W. O., & Ejovwokoghene E.F., *Occurrence of plasmid mediated fluoroquinolone resistance genes amongst enteric bacteria isolated from human and animal sources in Delta State, Nigeria,* **AIMS microbiology,** *7*(1), 2021, pg. 75.

Ayodele O.A., Deji-Agboola A.M., Faneye A.O., & Akinduti P.A., *Characterization and antibiotic susceptibility of E. coli O157: H7 in meat and fish sold in Major Ibadan Markets, Nigeria,* **American Journal of Biomedical Sciences,** *12*(2), 2020, pg. 99-106.

Babatunde O., Mercy F. & Adebola O.O., *Water quality and antimicrobial resistant profile of Escherichia coli isolated from dug wells used for domestic purposes*, **International Journal of Public Health,** *11*(1), 2022, pg. 129-137.

Bilal Aslam, Mohsin Khurshid, Muhammad Imran Arshad, Saima Muzammil, Maria Rasool, Nafeesa Yasmeen, Taif Shah, Tamoor Hamid Chaudhry, Muhammad Hidayat Rasool, Aqsa Shahid, Xia Xueshan & Zulqarnain Baloch *Antibiotic resistance: one health one world outlook,* **Frontiers in cellular and infection microbiology** 11, 2021, pg. 771510.

Bilal A., Khurshid M., Arshad M. I., Muzammil S., Rasool M., Yasmeen M. N. & Shah T. Antibiotic resistance: one health one world outlook, **Frontiers in cellular and infection microbiology** 11, 2021, pg. 771510.

Bushen A., Tekalign E. & Abayneh M., *Drug-and multidrug-resistance pattern of Enterobacteriaceae isolated from droppings of healthy chickens on a poultry farm in Southwest Ethiopia,* **Infection and drug resistance**, 2021 pp.2051-2058.

Chukwu E.E., Ibeh V.N. & Davies-Bolorunduro O.F., *Occurrence of Multi-Drug Resistant Listeria species in Faecal Samples of Poultry Chickens in Rural Farms in Lagos State, Nigeria,* **Advances in Infectious Diseases,** *11*(1), 2021, pg. 49-59.

de Mesquita Souza, Lim M. K., Marinho do Monte, Patrícia D. F., Givisiez E. N., Rodrigues L. B., de Freitas Neto O. C., Samuel Kariuki A. B., Júnior C. J., Br. de Oliveira, & Gebreyes

W. A., *Antimicrobial resistance in the globalized food chain: A One Health perspective applied to the poultry industry,* **Brazilian Journal of Microbiology** 2022, pg. 1-22.

Dike-Ndudim J. N., Ugwuegbu R. N., Amah H.C., Ndubueze C.W. & Anikwo E.S.,

Identity and Antibiogram of Bacterial Isolates from Owerri Modern Abattoir, Imo State, Nigeria, **Journal of Advances in Microbiology**, 2021, pg. 43-54.

Egbule O.S., Iweriebor B.C. & Odum E.I., *Beta-Lactamase-producing Escherichia coli*

isolates recovered from pig handlers in retail shops and Abattoirs in selected localities in Southern Nigeria: Implications for public health, **Antibiotics,** *10*(1), 2020, pg. 9.

Ekpunobi N.F., Adesanoye S., Orababa O., Adinnu C., Okorie C. & Akinsuyi S.,

Public health perspective of public abattoirs in Nigeria, challenges and solution, **GSC Biological and Pharmaceutical Sciences,** *26*(2), 2024, pg. 115-127.

Elena V.M., Miguel Galarde-López, Berta Carrillo-Quiróz, & Celia Mercedes Alpuche-

Aranda., *Antimicrobial resistance: one health approach,* **Veterinary world**, 15(3), 2022, pg. 743.

El-Ghany, W.A.A., *Staphylococcus aureus in poultry, with special emphasis on methicillin-*

resistant strain infection: a comprehensive review from one health perspective. 2021.

Esumirhewe J C., Cabal-Rosel A., Allerberger F., Springer B., & Ruppitsch W., *Genetic*

characterization of Escherichia coli and Klebsiella spp. from humans and poultry in Nigeria, **Access Microbiology,** *5*(7), 2023, pg. 000509-v4.

Fagbamila I.O., Ramon E., Lettini, A.A., Muhammad M., Longo A., Antonello K., Aworh,

Kwaga M.K., Abdu P.A., Umoh J.U., & Kabir J.A., *Assessing the mechanisms of multi-drug resistant non-typhoidal Salmonella (NTS) serovars isolated from layer chicken farms in Nigeria,* **Plos one,** *18*(9), 2023, pg. 0290754.

Falodun O.I. & Ameh A.A., *Multidrug resistance and virulence genes in Campylobacter*

isolates from poultry waste in Ibadan, Nigeria, **One Health Bulletin**, *4*(1), 2024, pg. 39-46.

Falodun O.I. & Waleola O.A., *Antibiotic resistance and virulence genes in Campylobacter species from pig and cattle samples in Ibadan, Nigeria,* **European Journal of Biological Research,** *14*(1), 2024, pg. 33-44.

Falodun O.I. & Ikusika E.O., *Extended spectrum and metallo-beta-lactamase Pseudomonas species from poultry and piggery waste,* **MicroMedicine,** *7*(2), 2019, pg. 37-45.

Falodun O.I., Buekor N.G. & Victor A., *Multidrug-resistant Enterococcus and coagulase-negative Staphylococcus isolates from poultry farms and nearby wells in Ibadan, Nigeria and their resistant genes*, **One Health Bulletin**, *4*(3), 2024, pg. 117-123.

Fasanmi, O.G., Adekunle, O.F., Okuneye, O.J., Akanbi, I.O., Adegor, E.C., Agbato, O.A., Lamidi, B.K., Emikpe, O.O., Owolabi, O.T., Olusegun, O.B. & Oyedepo M.O., *Evaluation of bacterial load and Multi-drug Antibacterial resistant Salmonella and Clostridium isolates from contaminated tools used in butcheries around Ibadan,* **Mathews Journal of Veterinary Science**, *7*(6), 2023, pg. 1-8.

Fashae K. & Hendriksen R.S., *Diversity and antimicrobial susceptibility of Salmonella enterica serovars isolated from pig farms in Ibadan, Nigeria,* **Folia microbiologica**, *59*, 2014, pg. 69-77.

Fasiku O.S., Omotosho O.O., Ajulo S.O., Adesanwo E.E., Oni O.D., Awoyele A.A., Okunlade O.A. & Adetunji V.O., *Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus in Pigs in Ibadan, Nigeria,* **In Medical Sciences Forum**, Vol. 24 (1), November 2023, pg. 8, MDPI.

Gaddafi M. S., Yakubu Y., Bello M. B., Bitrus A. A., Musawa A. I., Garba B., Lawal H., Aliyu M. A., Barka S. A., & Emeka A. J., "*Occurrence and antibiotic resistance profiles of Methicillin-resistant (MRSA) in layer chickens in Kebbi, Nigeria*." **Folia Veterinaria,** 66(2), 2022, pg. 37-45.

Gado D.A., Ehizibolo D.O., Meseko C.A., Anderson N.E. & Lurz, P.W., *Review of emerging and re-emerging zoonotic pathogens of dogs in Nigeria: Missing link in one health approach*, **Zoonotic Diseases**, *3*(2), 2023, pg. 134-161.

Hickman R. A., Leangapichart T., Lunha K., Jiwakanon J., Angkititrakul S., Magnusson U., Sunde M. & Järhult J.D., "*Exploring the antibiotic resistance burden in livestock, livestock handlers and their non-livestock handling contacts: a one health perspective,"* **Frontiers in Microbiology,** 12, 2021, pg. 651461.

Ibrahim D.D., Enem S.I., Egwu G., Dantong D., Jolayemi K.O. & Gaddafi M.S., *An emerging zoonosis: molecular detection of multidrug-methicillin resistant Staphylococcus aureus from butchers' knives, livestock products and contact surfaces,* **Veterinary Research Communications,** *48*(3), 2024, pg. 1697-1705.

Igbinosa I.H., Amolo C.N., Beshiru A., Akinnibosun O., Ogofure A.G., El-Ashker M., Gwida M., Okoh A.I. & Igbinosa E.O., *Identification and characterization of MDR virulent Salmonella spp. isolated from smallholder poultry production environment in Edo and Delta States, Nigeria,* **PloS One,** *18*(2), 2023, pg. 0281329.

Iwu C.J., *Prevalence and antibiogram of some swine associated Shiga toxin producing Escherichia coli Serogroups and Salmonella species in Nkonkobe Municipality, Eastern Cape Province, South Africa* **(Doctoral dissertation, University of Fort Hare),** 2015.

Jolapamo O.T., & Osatoyinbo O.O., *Isolation, Identification and Antibiogram of Bacterial Pathogens Gotten from Cow Meat Obtained from Different Market Sources,* **Funksec here,** *5*(2), 2023, pg. 72-79.

Kamweli A. M., Nilsson P., Egyir B., Owusu F.A., & Hendriksen R.S., "*Rare serovars of non-typhoidal Salmonella enterica isolated from humans, beef cattle and abattoir environments in Nigeria,"* **Plos one,** 19(1), 2024, pg. 0296971.

Kim, Dae-Wi, & Chang-Jun Cha. "*Antibiotic resistome from the One-Health perspective: understanding and controlling antimicrobial resistance transmission*," **Experimental & molecular medicine,** 53(3), 2021, pg. 301-309.

Kwaghe A.V., Okomah D.E., Aworh D.E., Awosanya E., Umeokonkwo C.D., Yarai E., Ameh C. & Kabir J., *Magnitude and trends of ruminants, Pigs and poultry diseases in Taraba State, Nigeria, 2013-2017: implications for public health.* **PAMJ-One Health,** *2*(20), 2020.

Lawal O.U., Adekanmbi O.A. & Adelowo O.O. *Occurrence of methicillin-resistant staphylococci in the pig-production chain in Ibadan, Nigeria*, **The Onderstepoort Journal of Veterinary Research,** *88*(1), 2021, pg.1959.

Ling J., Pruden A., Boehm A. B., Alvarez P., Raskin L., Kohn T., & Li X., *Integrating environmental dimensions of "One Health" to combat antimicrobial resistance: Essential research needs,* **Environmental Science & Technology,** 56(21), 2022, pg. 14871-14874.

Mamfe L.M., Akwuobu C.A. & Ngbede E.O., *Phenotypic detection, antimicrobial susceptibility and virulence profile of staphylococci in the pig production setting, Makurdi, Nigeria,* **Access Microbiology,** *3*(12), 2021, pg. 000293.

Matar, Ghassan M., Antoine Andremont & Wael Bazzi, "*Combating antimicrobial resistance-a one health approach*," **Frontiers in Cellular and Infection Microbiology,** 9, 2020, pg. 458.

Medugu N., Aworh M.K., Iregbu K., Nwajiobi-Princewill P., Abdulraheem K., Hull D.M., Harden L., Singh P., Obaro S., Egwuenu A. & Thakur S., *Molecular characterization of multi drug resistant Escherichia coli isolates at a tertiary hospital in Abuja, Nigeria*, **Scientific reports**, *12*(1), 2022, pg. 14822.

Monger X. C., Gilbert A., Saucier L. & Vincent A.T., "*Antibiotic resistance: From pig to meat."* **Antibiotics,** 10(10), 2021, pg. 1209.

Muleme J., Kankya C., Munyeme M., Musoke D., Sempebwa J.C., Isunju R.

Wambi J.B., Balugaba B.E., Sekulima T., Mugambe R.K. & Cadmus S., *Phenotypic Characterization and Antibiograms of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolated at the Human-Animal-Environment Interface Using a One Health Approach Among Households in Wakiso District, Uganda,* **Infection and Drug Resistance,** 2023, pg. 2203-2216.

Ndahi A.M., Fagbamila I.O., Adebiyi I., Ekeng E., Adeleke S., Adetunji V., Hendriksen R.S.,

Ryan C., Patel, H., Chisnal T. & Verner-Jeffreys D., *Establishing a One Health AMR Community of Practice in Nigeria,* **One Health Cases**, 2023, pg. 20230030.

Nwankwo I. *Epidemiology of Animal and Human Campylobacter Species Infections in Nigeria:*

A Retrospective Insight and the Need for One Health Approach in the Prevention and Control, **Veterinary Sciences and Practices,** *18*(1), 2023, pg. 1-11.

Nwosu, U.O., Ibiam F.A, Amadi-Ibiam C.O., Iroha C.S., Edemekong C.I., Peter I.U. &

Iroha I.R. *Fecal carriage of extended spectrum beta-lactamase and fluoroquinolone resistant gene in non-typhoidal Salmonella enterica isolates from food-producing animals and humans,* **Journal of Drug Delivery & Therapeutics,** *13*(9), 2023.

Ojo O.E., Amosun E.A., Opebiyi O.O., Oyekunle M.A., Dipeolu M.A. & Otesile E.B.,

Multidrug resistant enterohaemorrhagic Escherichia coli serogroups in the faeces of hunted Wildlife, Abeokuta, Nigeria, **Veterinaria Italiana,** *58*(2), 2022.

Ojo O.E., Iledare A.M., Amosun E.A. & Dipeolu M.A., *Antimicrobial use and detection of*

cefotaxime-resistant Enterobacteriaceae in the pig production chain, Ogun State, Nigeria, **Revue d'élevage et de médecine vétérinaire des pays tropicaux,** *72*(4), 2019, pg. 147-154.

Okorie-Kanu O.J., Anyanwu M.U., Ezenduka E.V., Mgbeahuruike A.C., Thapaliya D.,

Gerbig G., Ugwuijem E.E., Okorie-Kanu C.O., Agbowo P., Olorunleke S. & Nwanta J.A., *Molecular epidemiology, genetic diversity and antimicrobial resistance of Staphylococcus aureus isolated from chicken and pig carcasses, and carcass handlers*, **Plos one**, *15*(5), 2020, pg. 0232913.

Okunlade, A.O., Ogunleye A.O. & Ajuwape A.T.P., *Detection of mecA gene in oxacillin-*

resistant Staphylococcus aureus isolated from cattle, pigs, abattoir workers in two municipal abattoirs and their antibiotic profiles, **International Journal of Environmental Studies**, *81*(4), 2024, pg. 1813-1822.

Oloso N.O., Fagbo S., Garbati M., Olonitola S.O., Awosanya E. J., Aworh M.K., Adamu H., Odetokun I.A. & Fasina F.O., *Antimicrobial resistance in food animals and the environment in Nigeria: A review,* **International journal of environmental research and public health,** *15*(6), 2018, pg. 1284.

Olowe O.A., Adefioye O.J., Ajayeoba T.A., Schiebel J., Weinreich J., Ali A., Burdukiewicz M., Rödiger S. & Schierack P., *Phylogenetic grouping and biofilm formation of multidrug resistant Escherichia coli isolates from humans, animals and food products in South-West Nigeria,* **Scientific African,** *6*, 2019, pg. 00158.

Olubisose E.T., Ajayi A., Adeleye A.I. & Smith S.I., *Molecular and phenotypic characterization of efflux pump and biofilm in multi-drug resistant non-typhoidal Salmonella Serovars isolated from food animals and handlers in Lagos Nigeria,* **One Health Outlook**, *3*, 2015, pg.1-8.

Omitola O.O. & Taylor-Robinson A.W., *Emerging and re-emerging bacterial zoonoses in Nigeria: current preventive measures and future approaches to intervention*, **Heliyon,** *6*(6) 2020.

Omotosho O., Ukatu, S., Okandeji M., Okunlade O., Adeola A. & Emikpe B. *Antimicrobial Resistance Profile of Aerobic Nasal Microflora of Nigerian Indigenous Pigs,* **Alexandria Journal of Veterinary Sciences,** *76*(1), 2023.

Orum T.G., Ishola O.O., & Adebowale O.O., *Occurrence and antimicrobial susceptibility patterns of Salmonella species from poultry farms in Ibadan, Nigeria,* **African Journal of Laboratory Medicine,** *11*(1), 2022, pg. 1606.

Raufu I.A., Ahmed O.A., Aremu A., Chen J.C., Ameh J.A., Timme R.E, Hendriksen R.S. & Ambali A., *Whole Genome and Phenotypic Characterization of Novel QnrB19-*

positive Salmonella Nigeria serovars from Food Animals in Ilorin, North-central, Nigeria. 2021.

Raufu I.A., Ahmed O.A., Aremu A., Ameh J.A., Timme R.E., Hendriksen R.S. & Ambali A.G.,

Occurrence, antimicrobial resistance and whole genome sequence analysis of Salmonella serovars from pig farms in Ilorin, North-central Nigeria, **International Journal of Food Microbiology,** *350*, 2021, Pg. 109245.

Raufu I.A., Ahmed O.A., Aremu A., Ameh J.A., Timme R.E., Hendriksen R.S. & Ambali A.,

Antimicrobial and genomic characterization of salmonella Nigeria from pigs and poultry in Ilorin, North-Central, Nigeria, **Journal of infection in developing countries**, *15*(12), 2021, pg. 1899 -1909.

Romiluyi B., Itaman V.O, Odumosu B., Adeleye I.A. & Smith S.I., *Molecular Characterization*

of Extended Spectrum Beta lactamase Producing Enterobacteriaceae Isolated from Duck Droppings, **Nigerian Journal of Microbiology,** 2023.

Soma D., Bonkoungou I.J.O., Garba Z., Diarra F.B.J., Somda N.S., Nikiema M.F.M. , Bako E.,

Sore E., Sawadogo S., N., Barro N. & Haukka K. *Extended-Spectrum Beta-Lactamase-Producing and Multidrug-Resistant Escherichia coli and Klebsiella spp. from the Human–Animal–Environment Interface on Cattle Farms in Burkina Faso*, **Microbiology Research**, *15*(4), 2024, pg. 2286-2297.

Sunil P., Shrestha P. & Adhikari. B., *Antimicrobial use in Food Animals and Human Health: Time*

to implement 'One Health'approach, **Antimicrobial Resistance & Infection Control,** 9, 2020, pg. 1-5.

Taylor-Robinson A.W. & Omitola O.O., *Emerging and Re-Emerging Bacterial Zoonoses: A*

Nigerian Perspective on Control, Prevention and Intervention. In Zoonosis of Public Health Interest, **IntechOpen,** 2022.

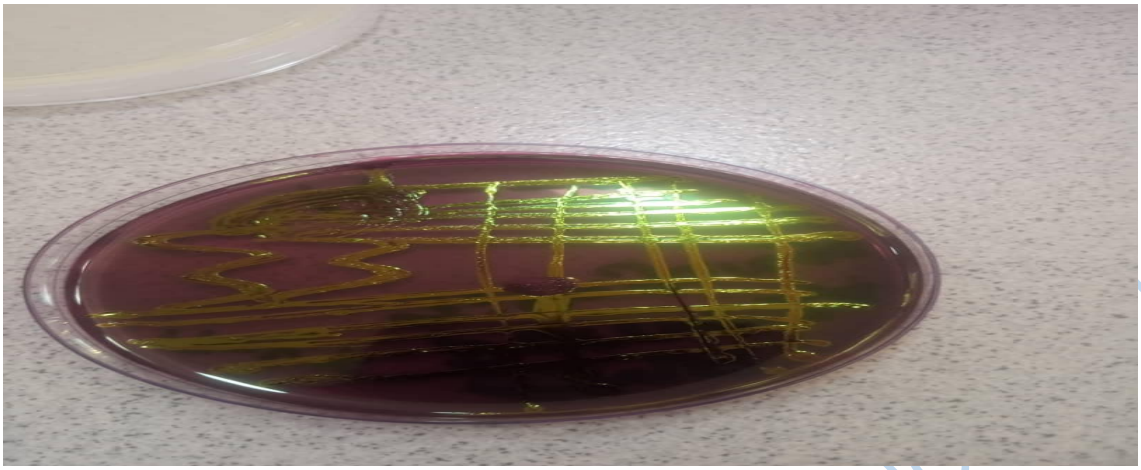
Tiamiyu A.M., Okocha R.C., Adesina I.A., Okon, E.M., Olatoye, I.O. & Adedeji O.B.,

Antimicrobial resistance: A significant public health issue of both human and veterinary

Concern, **Open Health,** *5*(1), 2024, pg. 20230046.

Uzairue L.I., & Shittu O.B., *Salmonella enterica Transmission and Antimicrobial Resistance Dynamics across One-Health Sector*. **In Salmonella-Perspectives for Low-Cost Prevention, Control and Treatment**. **IntechOpen,** 2023.

Appendices

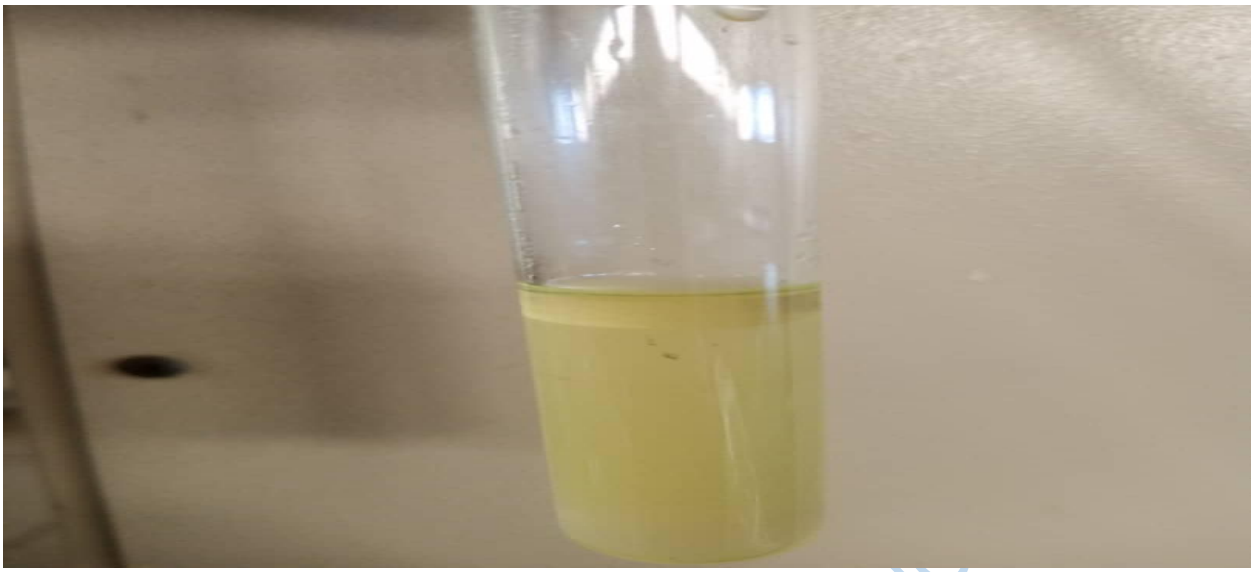


Appendix 1: Pure culture of *E. coli* on Eosine Methylene Blue Agar

Source Author Field work, 2025

Appendix 2: Collection of samples from the pigs

Source Author Field work, 2025

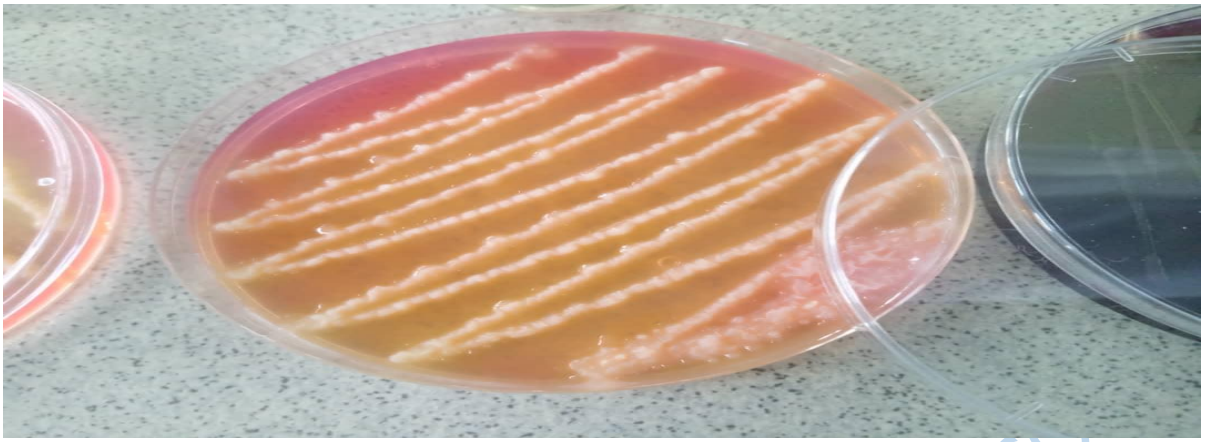


Appendix 3: Indole test showing negative result

Source Author Field work, 2025

Appendix 4: Indole test showing positive result

Source Author Field work, 2025



Appendix 5: Pure culture of *Staphylococcus aureus* growing on Manitol Salt Agar

Source Author Field work, 2025

Appendix 6: MacCartney bottles containing the samples in broth

Source Author Field work, 2025

Appendice 7: Methyl red test showing negative result

Source Author Field work, 2025

Appendice 8: Methyl red test showing positive result

Source Author Field work, 2025

Appendix 9: Antibody Disk on Muller Hilton Agar

Source Author Field work, 2025

Bio-data

A. Personal Data:

1. **Full Name**: Adeyemi Samson ABEEB

2. Address: Plot 9 Block 15–19 Oluayeni Layout Ashipa, Odo Ona Elewe, Orita Challenge, Ibadan.

3. Email: honsay2001@gmail.com

4. **Date and Place of Birth**: 11th March, 1979. Ibadan

5. **Name and Address of Next of Kin**: Abeeb Titilayo Olabisi, Plot 9 Block 15 – 9 Oluayeni Layout Ashipa, Odo Ona Elewe, Orita Challenge, Ibadan.

B. Educational Background

Educational Institutions Attended with Dates and Qualifications:

i. Primary Education

I.D.C. Primary School, Ibadan.	1984 - 1990

ii. Secondary Education

Eyinni High School, Ibadan.	1991 - 1996

iii. Higher Educational Institutions

Federal Polytechnic, Offa.	1999 - 2003
Federal Polytechnic, Offa.	2005 - 2006
Lead City University, Ibadan.	2022-

Qualifications Obtained

Primary School Leaving Certificate	1990
Senior Secondary School Certificate	1996
OND in Science Laboratory Technology	2003
HND in Science Laboratory Technology (Bio/Microbiology Option)	2006
MSc. Medical Microbiology	2025

C. Working Experience with Dates

Laboratory Technologist. 2021 -Till Date

D. Awards and Fellowship

Nil

E. Membership of Academi Professional Bodies

i. NISLT- Nigeria Institute of Science Laboratory Technologist

F. Major conferences/Workshops Attended

3rd International conference. FASCON, Lead City University, Ibadan, Titled: Translational Research in Science and Technology for Sustainable Development Circa COVID-19 Era. 2nd – 4th November, 2022.

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G. Publication

Antimicrobial Resistance in Nigeria and the One Health Approach to Antimicrobial Stewardship: A Review Umezurike ET*, **Abeeb AS**, Ajadi ST and Alimi BF Journal of Life Sciences Research and Reviews. Volume 2 Page 1-6

Mycotoxin Content of Fungi Contaminated Sorghum Sold in Selected Markets in Ibadan, Nigeria. B. A. Bamkefa*, O. A. Fatoki, O. Abiola-Olagunju, A. Duyilemi-Olaniyi, **A. S. Abeeb**, K. O. Adediran and O. O. Ajogbeje. Journal of Advances in Microbiology Volume 23, Issue 7, Page 26-31, 2023; Article no. JAMB.102168 ISSN: 2456-7116

Microbial Profile of Smoked Fish Sold in Selected Markets in Ibadan Metropolis, Oyo State. Umezurike, E.T., K.O. Adegbehingbe, **A.S. Abeeb**, O. Sindiku, A. P. Effiong, V.A. Melle, B. F. Alimi, and S.T. Ajadi. 2024. "*Microbial Profile of Smoked Fish Sold in Selected Markets in Ibadan Metropolis, Oyo State, Nigeria*". Journal of Advances in Microbiology 24 (12):128-38. https://doi.org/10.9734/jamb/2024/v24i12878.

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The University Compliance Certification

This is to certify that the Thesis by **Abeeb Adeyemi Samson** with Matric No **LCU/PG/004033** in the department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

_____ _____

Name and Signature **Date**