PHENOTYPIC DETECTION OF GRAM-NEGATIVE BACTERIAL ISOLATES FROM FAECES OF DOMESTIC RABBITS (*Oryctolagus cuniculus*): IMPLICATIONS FOR ANTIBIOTIC RESISTANCE IN PUBLIC HEALTH

BY

STEPHANIE OTOKUNEFOR
LSC2104020

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY, EDO STATE,
NIGERIA.

NOVEMBER, 2025.

PHENOTYPIC DETECTION OF GRAM-NEGATIVE BACTERIAL ISOLATES FROM FAECES OF DOMESTIC RABBITS (*Oryctolagus cuniculus*): IMPLICATIONS FOR ANTIBIOTIC RESISTANCE IN PUBLIC HEALTH

BY

STEPHANIE OTOKUNEFOR
LSC2104020

A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE. IN UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by Stephanie Otokunefor in the Department of Microbiology, Faculty of Life Sciences, and University of Benin, Benin City under my supervision.

_____ _____

Prof. (Mrs.) O.I. Enabulele **Date**
(Supervisor)

_____ _____

Prof. (Mr.) E. O. Igbinosa **Date**
(Head of Department)

DEDICATION

This project is dedicated to Almighty God and my supervisor Prof. (Mrs.) O.I. Enabulele, my parents and siblings for their support and encouragement during my journey,

ACKNOWLEDGEMENTS

I would like to thank God for his continued grace and guidance, for how good he has been to me throughout my schooling and for his protection throughout the research project. I would also like to express my sincere thanks to my supervisor Prof. (Mrs.) O.I. Enabulele and my sincere thanks to Dr. A.S. Aziegbemhin for his continuous guidance, support and knowledge imparted, it was really great working under you sir. I would like to thank my fellow students, friends and colleagues for the time we spent together and all the memories it brought. Special thanks to the head of department Prof. (Mr.) E. O. Igbinosa and other staff of the Department of Microbiology for their support. I extend my heartfelt gratitude to my parents Mr. and Mrs. Gabriel Otokunefor and My Siblings.

Finally, I acknowledge all authors and researchers whose works were referenced in this project, your contributions to the field of microbiology have been invaluable in shaping my research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Rabbit gastrointestinal tracts harbour a variety of bacteria that are shed in stool and some are beneficial while others are pathogenic. Fifteen (15) rabbit stool samples were collected from three different farms (University of Benin farm, Okene Farm and Animal house) with sterile universal bottles and samples were immediately transported to the laboratory for bacteriological analysis. Total heterotrophic bacterial counts and culture were carried out using standard microbiological techniques. Bacterial identification was also carried out using selective media and various biochemical tests. Phenotypic virulence factors (haemolysin, gelatinase production, serum resistance and capsule formation assays) were carried out on the bacterial isolates. Antibiotic susceptibility was carried out using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar. Results showed that Animal House had the highest bacterial load ($12.9 \times 10^6 \pm 1.4$ CFU/g) while University of Benin Farm the lowest ($2.1 \times 10^6 \pm 1.4$ CFU/g) ($p < 0.001$). The bacteria isolated include, *Lactobacillus* sp. (26%), *Neisseria* sp. (20%), *Staphylococcus aureus* (20%), *Bacillus* sp. (13%), *Escherichia coli* (13%) and *Salmonella* sp. (6%). *Staphylococcus aureus*, *Bacillus* sp., *E. coli*, and *Salmonella* sp. exhibited β-haemolysis, gelatinase activity, serum resistance and capsule formation, while *Lactobacillus* sp. and *Neisseria* sp. lacked these virulence factors. All isolates were susceptible to levofloxacin, ciprofloxacin, rifampicin and ceftazidime but resistant to gentamicin, cefuroxime, streptomycin, azithromycin, amoxicillin and erythromycin. Rabbit stool contains both commensal and opportunistic pathogens carrying resistant bacteria.

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Rabbits (*Oryctolagus cuniculus*) play an important role in human society, serving as sources of meat and essential laboratory models. As pets, their popularity has grown in recent decades, particularly in Europe, North America and Asia where they are valued for their sociable nature and adaptability to domestic life (Skovlund *et al*., 2023). In livestock systems, rabbits are farmed primarily for meat production, a practice more common in Mediterranean and Asian countries but its recognition is increasing in other regions as a niche industry. Rabbit meat production often relies on intensive farming practices, in which antimicrobial agents are frequently employed not only for therapeutic purposes but also for prophylaxis and growth promotion (Sun *et al*., 2024). Beyond the domestic and agricultural value, rabbits also serves as laboratory animals in biomedical research. Their physiological and immunological characteristics make them valuable models for vaccine development, infectious disease studies and toxicological assessments, thereby linking them directly to both veterinary and human health outcomes (Sun *et al*., 2024). This is a key factor that required the necessity of monitoring rabbit health and microbiota, not only for animal welfare but also for the prevention of zoonotic transmission of pathogens.

Faecal matter reflects the balance of the gut microbiota, which includes commensal and pathogenic microorganisms. Several Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica* and *Pseudomonas aeruginosa* are frequently detected in rabbit feces (Zhao *et al*., 2024). While many of these organisms are harmless, certain strains carry virulence genes that may predispose rabbits to gastrointestinal diseases, systemic infections or

asymptomatic carriage of zoonotic pathogens (da Silva *et al*., 2024). Rabbits have been identified as potential reservoirs of antimicrobial-resistant bacteria that could be transmitted to humans through direct contact, consumption of rabbit meat or environmental contamination via fecal material. Sun *et al*., (2024), reported that multidrug-resistant *E. coli* and *Klebsiella* strains in both pet and farmed rabbits are raising concerns about their role in the environmental circulation of resistant pathogens (Sun *et al*., 2024).

Globally, antimicrobial resistance (AMR) in Gram-negative bacteria is a critical public health challenge. Gram-negative pathogens such as *Enterobacteriaceae* and *Pseudomonas* spp. have demonstrated remarkable adaptability in acquiring and disseminating resistance genes, often mediated by plasmids and other mobile genetic elements (Hu *et al*., 2023). Rabbit harbor bacteria that are resistant to multiple antimicrobial classes, like tetracyclines, aminoglycosides, fluoroquinolones and β-lactams. Extended-spectrum β-lactamases (ESBLs) such as *blaCTX-M*, *blaTEM* and *blaSHV*, which significantly limit therapeutic options in both veterinary and human medicine (Jiang *et al*., 2024). Sun *et al*., (2024), reported that rabbit harbor bacteria with plasmid-mediated colistin resistance genes (*mcr-1*), a last-resort antibiotic often used to treat multidrug-resistant infections (Sun *et al*., 2024). Antimicrobial resistance and virulence factors are central to understanding the pathogenic potential of rabbit-associated Gram-negative bacteria. Genes encoding toxins, adhesins enhance the ability of pathogens to colonize hosts, evade immune defenses and cause systemic disease (Badagliacca *et al*., 2023). Enteropathogenic *E. coli* strains carrying *eae* and *stx* genes, cause diarrheal diseases in rabbits while extraintestinal pathogenic *E. coli* (ExPEC) often possess virulence determinants such as *fimH* and *pap* that facilitate urinary tract or bloodstream infections (Johnson and Nolan, 2019).

Aim and objectives

To determine the antimicrobial resistance patterns and virulence factors of Gram-negative bacteria from rabbit stool

The objectives of this study were to;

i. isolate and identify the bacteria present in rabbit stool samples
ii. determine the susceptibility pattern of the bacteria isolates from rabbit stool samples
iii. determine the virulence factors (Gelatinase, Capsule, Haemolysis and Serum resistance) of bacteria isolates from rabbit stool samples

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) is one of the biggest threats to health, development and security in the 21st century. It happens when bacteria, viruses, fungi and parasites change in ways that make medicines less effective. While resistance can occur naturally, its rapid spread today is mainly caused by human actions. Overuse and misuse of antibiotics in hospitals, agriculture and communities have fueled the rise of resistant germs across the world. This problem has moved beyond science alone to become a social, economic and political crisis. The World Health Organization (2015) has warned that AMR could lead to a "post-antibiotic era," where even simple infections could once again be deadly. Holmes *et al*. (2016) also describe AMR as a global emergency rooted in medical practices, economic inequality and environmental factors.

Global statistics highlight the seriousness of this crisis. In 2019, bacterial AMR caused about 1.27 million deaths directly and was linked to nearly 4.95 million deaths worldwide (Murray et al., 2022). Western Sub-Saharan Africa has some of the highest death rates, estimated at around 27.3 deaths per 100,000 people. These figures show that AMR is not only a health concern but also a major global challenge that cuts across countries and regions.

Regionally, the African continent bears a heavy burden of AMR. In 2021, bacterial AMR was linked to around 4.71 million deaths in Africa, making it the hardest-hit region compared to others worldwide. The problem is even worse for children: in 2022, over 3 million children globally died from AMR-related infections, with more than 650,000 of these deaths occurring in Africa alone (World Health Organization (WHO), 2024). This shows how AMR is not only a health crisis but also a threat to child survival in low-resource settings.

In Nigeria, the scale of AMR is equally alarming. In 2019, the country ranked 20th worldwide for AMR-related deaths, with about 263,400 deaths linked to bacterial AMR. Recent government reports estimate that more than 65,000 deaths in Nigeria are directly caused by AMR each year. The economic impact is also significant, with AMR reducing Nigeria's gross domestic product (GDP) by about 7% and lowering livestock productivity by 11%. Despite this, public knowledge remains poor: more than half of Nigerians surveyed had heard of "antibiotic resistance," but only about 8% had a good understanding of it, while over 30% reported using antibiotics without a prescription (World Health Organization (WHO), 2024). these statistics prove that AMR is not a future concern but a present reality. It is already causing widespread deaths and deep economic losses, especially in low- and middle-income countries where health systems are weaker. The evidence highlights the urgent need for global action while also showing that the burden is uneven. Understanding the scale of AMR at local, regional and global levels is crucial for designing effective policies, directing resources and protecting public health.

2.2 Causes of Antimicrobial Resistance

The causes of AMR are deeply interconnected, involving behavioral, environmental and systemic factors. In human health, inappropriate use of antibiotics is a dominant driver. This includes unnecessary prescriptions for viral infections, incomplete treatment courses by patients and empirical use of broad-spectrum antibiotics in place of targeted therapy. Research in several countries demonstrates that up to half of all antibiotic prescriptions are unnecessary, fueling selection pressure for resistant bacteria (Holmes *et al*., 2016).

Agricultural practices also play a significant role. Antibiotics are commonly used not only to treat infections in livestock but also to promote growth and prevent disease in crowded farming systems. These practices expose large microbial populations to subtherapeutic doses of

antibiotics, encouraging resistance genes to develop and spread. Resistant bacteria from animals can be transferred to humans through food chains, direct contact or environmental contamination. O'Neill (2016) emphasized that the extensive use of antibiotics in food production represents a "critical breach" in antimicrobial stewardship.

Environmental contamination is another underestimated factor. Pharmaceutical waste from drug manufacturing plants, hospital effluent and agricultural runoff introduce active antibiotic compounds and resistant organisms into soil and water systems. These environments then serve as reservoirs and mixing grounds for resistance genes, which can transfer between species through horizontal gene transfer. Recent studies suggest that the environmental "resistome" is an increasingly important source of clinical resistance (Holmes *et al*., 2016).

Low- and middle-income countries (LMICs) face unique challenges that accelerate AMR. Weak laboratory infrastructure, limited access to diagnostics and unregulated antibiotic sales makes inappropriate antibiotic use more common. Over-the-counter availability of antibiotics without prescriptions, a practice still common in many parts of Africa and Asia, contributes heavily to misuse (Kakkar *et al*., 2018). In addition, global travel and trade mean that resistant organisms can quickly cross borders, turning local resistance problems into international crises.

2.3 The Importance of Antimicrobial Resistance

The consequences of AMR are profound, spanning health, economics and development. Clinically, resistant infections are associated with longer hospital stays, higher medical costs and increased mortality. Murray *et al*. (2022) reported that in 2019 alone, antimicrobial resistance was directly responsible for 1.27 million deaths and associated with nearly 5 million deaths worldwide. These figures place AMR among the leading causes of death globally, surpassing HIV/AIDS and malaria.

Economically, the burden of AMR is staggering. Resistant infections demand expensive second- or third-line drugs, often with greater toxicity and lower availability. Longer hospital stays and repeated treatments drive up healthcare costs. The World Bank (2017) estimated that by 2050, unchecked AMR could cause global GDP losses of up to 3.8%, disproportionately affecting low-income countries. Beyond healthcare, AMR poses a threat to food security, as it undermines livestock production and to global development, as it exacerbates poverty and inequality.

Equally important is the impact of AMR on medical progress. Modern healthcare relies heavily on antibiotics to safeguard procedures such as organ transplantation, chemotherapy and major surgeries. Without effective antibiotics, these life-saving interventions become dangerously risky. Thus, AMR does not simply affect the treatment of infectious diseases but undermines the foundation of contemporary medicine (Wellington *et al.,* 2013).

2.4 Strategies to Combat Antimicrobial Resistance

Addressing AMR requires a comprehensive and coordinated response, often described under the "One Health" framework, which integrates human, animal and environmental health. The WHO's Global Action Plan (2015) outlines several priority areas.

Firstly, antimicrobial stewardship is central. This involves optimizing antibiotic prescribing through guidelines, education and monitoring. Hospitals implementing stewardship programs have reported reductions in antibiotic use and lower rates of resistant infections (Baur *et al*., 2017). In LMICs, stewardship efforts must be adapted to local contexts, where diagnostic tools and trained personnel may be limited.

Secondly, infection prevention and control (IPC) measures are vital. Improved hygiene in hospitals, proper handwashing, sterilization of equipment and vaccination can significantly reduce infection rates, thereby reducing the need for antibiotics. Expanded access to vaccines,

such as pneumococcal and influenza vaccines, has already been shown to reduce antibiotic use in children and vulnerable populations (Laxminarayan *et al*., 2016).

Thirdly, surveillance systems must be strengthened. Monitoring resistance trends helps identify emerging threats and guides interventions. Programs such as the Global Antimicrobial Resistance Surveillance System (GLASS) by WHO aim to standardize data collection across countries. However, disparities in diagnostic capacity remain a major obstacle, especially in low-resource settings.

Fourth, regulatory and policy measures are essential. Governments must enforce laws to prevent over-the-counter sales of antibiotics, regulate agricultural use and incentivize pharmaceutical companies to invest in new drug development. The challenge of a dwindling antibiotic pipeline has been widely recognized. Push-and-pull incentives, such as grants for early-stage research and market entry rewards, are increasingly recommended to stimulate innovation (O'Neill, 2016).

Finally, research and development must focus not only on new antibiotics but also on alternative approaches. Promising strategies include bacteriophage therapy, antimicrobial bacteria, probiotics and immune-based treatments. Advances in rapid diagnostics can also revolutionize treatment by ensuring antibiotics are only used when necessary and that the right drug is chosen for the right infection (Tacconelli *et al*., 2018).

2.5 Antimicrobial Resistance in Gram-Negative Bacteria

Antimicrobial resistance (AMR) is particularly severe in Gram-negative bacteria, which are now recognized as some of the most dangerous pathogens in both hospital and community settings. Compared to Gram-positive organisms, Gram-negative bacteria often show higher rates of resistance. This is largely due to their unique cell wall structure, which includes an outer membrane that acts as a barrier to many antibiotics. Global surveillance studies have reported that multidrug-resistant Gram-negative infections, such as those caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are increasing at alarming rates, particularly in low- and middle-income countries where infection control and antibiotic stewardship programs are limited (Murray *et al*., 2022). In fact, the World Health Organization has listed several of these Gram-negative pathogens as "critical priority" organisms for research and development of new antibiotics (WHO, 2017).

The higher prevalence of resistance in Gram-negative bacteria compared with Gram-positive ones is explained by both biological and environmental factors. Biologically, Gram-negative organisms naturally possess more defense mechanisms, including their protective outer membrane and a wide range of resistance genes that can be easily transferred between species through plasmids and other mobile genetic elements. Environmentally, poor sanitation, uncontrolled antibiotic use in humans and animals and inadequate hospital infection control further amplify their spread. As a result, Gram-negative bacteria are often resistant to multiple classes of antibiotics at once, leaving clinicians with limited treatment options.

The mechanisms of resistance in Gram-negative bacteria are diverse and often occur simultaneously, making them particularly difficult to treat. One major mechanism is enzymatic degradation of antibiotics. This includes the production of β-lactamases such as extended-

spectrum β-lactamases (ESBLs), carbapenemases and metallo-β-lactamases, which can break down penicillins, cephalosporins and even carbapenems—the drugs of last resort. Another key mechanism involves efflux pumps, which actively expel antibiotics from the bacterial cell before they can reach their target. These pumps often contribute to multidrug resistance because they can remove a wide variety of antibiotics simultaneously.

In addition, Gram-negative bacteria can undergo target modification, where mutations alter the binding sites of antibiotics, reducing their ability to act. For example, mutations in DNA gyrase and topoisomerase IV lead to fluoroquinolone resistance, while modifications of ribosomal subunits can reduce susceptibility to aminoglycosides. Another crucial mechanism is reduced permeability of the bacterial cell wall. Changes in porin proteins in the outer membrane can prevent antibiotics, such as β-lactams and carbapenems, from entering the bacterial cell, further contributing to resistance.

2.6 Rabbits as Reservoirs of Gram-Negative Bacteria

Rabbits carry a variety of Gram-negative bacteria in their gut microbiota. These microorganisms support digestion and immunity, but some can act as pathogens and affect animal and human health (Massacci *et al*., 2018). The balance between normal and harmful strains can be disrupted by antibiotic use, diet or stress, leading to an overgrowth of resistant bacteria (Sanchez *et al*., 2019).

The main Gram-negative bacteria found in rabbit stool include *Escherichia coli*, *Klebsiella spp.*, *Salmonella spp.* and *Pseudomonas spp.* (Alvarez *et al*., 2020). These may exist as part of normal flora but can also cause disease, especially in intensive breeding systems with poor hygiene. Resistant strains, such as ESBL-producing *E. coli* and carbapenem-resistant *Klebsiella*, have been reported in both farmed and pet rabbits (Liu *et al*., 2020).

These bacteria can spread from rabbits to humans through direct contact, contaminated rabbit meat or environmental release into soil and water (Pirolo *et al*., 2019). In small-scale farming systems, the risk of environmental spread is higher, which can add to community-wide antimicrobial resistance (Fasanella *et al*., 2020). Rabbits are also used as models to study enteric pathogens and resistance. Their gut microbiota has similarities to humans, making them useful for research on host–pathogen interactions and the effects of antibiotics (Segura *et al*., 2021). Studies in rabbits have provided information on the transmission and disease patterns of *Salmonella* and enteropathogenic *E. coli* (Martin-Pelaez *et al*., 2020). Compared with other animals, rabbits share similarities with poultry and livestock in carrying resistant zoonotic bacteria, though their microbiota and farming practices affect prevalence and diversity. Unlike rodents, which are mainly environmental reservoirs, rabbits also contribute through meat consumption, which creates a more direct food safety concern (Pirolo *et al*., 2019).

2.8 Detection of Antimicrobial Resistance

2.7.1 Phenotypic detection methods

2.7.1.1 Disk Diffusion (Kirby–Bauer Method)

The disk diffusion test is the most widely applied phenotypic assay for AMR detection. Developed in the 1960s, it remains a cornerstone of susceptibility testing in resource-limited laboratories. In this method, standardized bacterial inoculum are spread onto Mueller-Hinton agar plates. Antibiotic-impregnated paper disks are placed on the agar and during incubation, antibiotics diffuse radially. Zones of inhibition form where bacterial growth is suppressed. These zones are measured and compared against standardized interpretive criteria from CLSI (Biemer, 1973; CLSI, 2020).

Strengths

- Low cost and simplicity make it accessible worldwide.
- Multiple antibiotics can be tested simultaneously.
- Easy to standardize and interpret.
- Suitable for routine diagnostic labs and surveillance programs.

Limitations

- Provides only qualitative susceptibility categories (*susceptible, intermediate, resistant*) but not exact MIC values.
- Antibiotics with poor diffusion properties (e.g., colistin) cannot be reliably tested.
- Environmental factors (agar thickness, inoculum density, incubation conditions) can influence results.
- Less accurate for fastidious organisms or those requiring special growth conditions.

Despite these limitations, disk diffusion remains the backbone of AMR testing in many low- and middle-income countries (LMICs) due to its affordability and standardization by international bodies (Klein *et al*., 2018).

2.7.1.2 Minimum Inhibitory Concentration (MIC) Assays

The MIC represents the lowest concentration of an antimicrobial that inhibits visible bacterial growth. MIC testing can be performed by broth microdilution, broth microdilution or automated systems. Broth microdilution, where serial twofold dilutions of antibiotics are prepared in microtiter plates, is the reference standard recommended by CLSI (Wiegand *et al*., 2008).

Strengths

- Produces precise quantitative results, critical for guiding therapy.
- Considered the gold standard for determining susceptibility.

- Essential for pharmacokinetic/pharmacodynamic (PK/PD) modeling and antibiotic dosing decisions.
- Automated platforms (VITEK 2, MicroScan, BD Phoenix) allow high-throughput testing.

Limitations

- Technically demanding in manual form, requiring careful preparation and aseptic handling.
- More costly and resource-intensive compared to disk diffusion.
- Automated systems are expensive and may not be available in LMICs.
- Results may vary based on media composition and inoculum preparation.

MIC assays are particularly valuable for critical cases (septicemia, multidrug-resistant infections) where exact drug concentration data guide clinical decisions (Bush and Bradford, 2019).

2.7.1.3 E-test (Epsilometer Test)

The E-test is a hybrid method that combines the simplicity of disk diffusion with the quantitative precision of MIC testing. Plastic strips containing a predefined gradient of antibiotic concentrations are placed on an inoculated agar plate. After incubation, an ellipse of inhibition forms and the MIC is read where the ellipse intersects the scale on the strip (Jorgensen and Ferraro, 2009).

Strengths

- Provides both qualitative and quantitative data.
- Easier to use than broth dilution while still yielding MIC values.
- Useful for antibiotics with poor diffusion (glycopeptides, polymyxins).
- Effective for fastidious organisms that do not grow well in broth dilution systems.

Limitations

- More expensive per test than disk diffusion.
- Requires high-quality agar and careful strip placement.
- Less scalable than broth microdilution for high-throughput testing.
- Variability may occur compared with reference MIC values.

The E-test is often used as a confirmatory method, especially for critical antibiotics such as colistin, vancomycin and carbapenems, where accurate MIC determination is essential (Sun *et al*., 2019).

2.7.2 Genotypic detection methods

Genotypic methods for detecting antimicrobial resistance (AMR) have become essential in modern microbiology due to their ability to provide rapid, sensitive and specific identification of resistance determinants. Unlike phenotypic approaches, which depend on the growth of bacteria in the presence of antibiotics, genotypic methods target the genetic material of pathogens, allowing for earlier detection and detailed molecular characterization of resistance mechanisms (Jorgensen and Ferraro, 2009).

2.7.2.1 Polymerase Chain Reaction (PCR) for Specific Resistance Genes

Polymerase chain reaction (PCR) is one of the most widely applied molecular methods for detecting antimicrobial resistance (AMR) genes. It works by amplifying specific DNA sequences, enabling the identification of resistance determinants such as *blaCTX-M*, *blaNDM* and *tetA*, which are responsible for extended-spectrum β-lactamase production, carbapenem resistance and tetracycline resistance, respectively. PCR offers high sensitivity and specificity, providing rapid results that can guide clinical decision-making (Sun *et al*., 2019). Its simplicity and relatively low cost make it suitable for routine use in many laboratories. However, PCR is inherently

limited by its targeted nature: it can only detect genes that are already known and for which primers are designed. As a result, novel or uncharacterized resistance determinants remain undetected, leaving gaps in surveillance (Wiegand *et al*., 2008).

2.7.2.2 Whole-Genome Sequencing (WGS)

Whole-genome sequencing (WGS) has revolutionized the detection and monitoring of AMR by enabling comprehensive profiling of bacterial genomes. Unlike PCR, which focuses on individual genes, WGS provides a complete picture of all resistance determinants present, including known, novel and horizontally acquired genes. This method also reveals the genetic context of resistance, such as plasmids, integrons and transposons, which play crucial roles in the spread of resistance among bacterial populations (Murray *et al*., 2022). WGS is increasingly used in global surveillance programs to trace outbreaks, monitor the emergence of resistance and inform public health strategies (Klein *et al*., 2018). Despite its powerful capabilities, the routine application of WGS is still limited by cost, infrastructure requirements and the need for advanced bioinformatics expertise, particularly in low- and middle-income countries (Laxminarayan *et al*., 2020).

2.7.3 Strengths and Limitations of Genotypic Methods

Genotypic methods, particularly PCR and WGS, each offer unique strengths that complement phenotypic approaches. PCR is rapid, inexpensive and ideal for detecting clinically relevant resistance genes when quick results are needed to guide treatment. In contrast, WGS is more resource-intensive but provides unparalleled resolution in identifying both known and novel resistance determinants, making it indispensable for research and surveillance. A key limitation of both approaches is their inability to directly measure resistance expression; the presence of a gene does not always guarantee phenotypic resistance due to regulatory and environmental

factors (Jorgensen and Ferraro, 2009). Therefore, integrating genotypic methods with phenotypic testing remains essential for a complete understanding of AMR dynamics.

2.8 Virulence Factors in Gram-Negative Bacteria

Virulence factors are molecules or structures produced by bacteria that enable them to colonize, invade and cause disease in their hosts. In Gram-negative bacteria, these factors play critical roles in survival, pathogenicity and the ability to persist within diverse environments. They include toxins, enzymes, surface structures and secretion systems that collectively allow bacteria to evade host defenses, acquire nutrients and establish infection (Frieri *et al*., 2017). Importantly, virulence traits are not evenly distributed among all strains but are often associated with pathogenic lineages that threaten both human and animal health. The ability of Gram-negative bacteria to colonize and persist in host tissues is strongly dependent on their virulence factors. For instance, adhesins and pili enable bacteria to attach to host epithelial surfaces, while capsules and surface proteins help them resist immune clearance. Once colonization is achieved, toxins and enzymes disrupt host tissues, creating favorable niches for bacterial multiplication. These mechanisms also enhance bacterial survival by promoting resistance to environmental stresses and antimicrobial agents, further increasing the risk of persistent or recurrent infections (Prestinaci *et al*., 2015). Many virulence genes are located on plasmids, integrons or other mobile genetic elements that also carry resistance determinants. This genetic co-location means that antibiotic use can indirectly select for virulence traits, a phenomenon known as co-selection (Aslam *et al*., 2018). For example, plasmids encoding extended-spectrum β-lactamases (ESBLs) often also carry genes for toxins, adhesion or biofilm production. This dual advantage makes resistant strains more difficult to treat and more capable of causing severe infections.

2.8.1 Hemolysin Production

Hemolysins are pore-forming toxins secreted by several Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*. Their primary function is to lyse host red blood cells, thereby releasing iron and other nutrients essential for bacterial growth. In addition to nutrient acquisition, hemolysin activity damages host tissues and facilitates bacterial invasion, contributing to severe clinical manifestations such as sepsis and urinary tract infections (Bush and Bradford, 2019).

2.8.2 Capsule Formation

Capsule production is a key virulence mechanism in Gram-negative bacteria, allowing pathogens such as *Klebsiella pneumoniae* to resist phagocytosis and complement-mediated killing. The polysaccharide capsule creates a physical barrier between the bacterium and the host immune system, promoting long-term survival within the host (Logan and Weinstein, 2017). Capsulated strains are often associated with more severe infections and higher mortality rates compared to non-capsulated strains.

2.8.3 Serum Resistance

Serum resistance refers to the ability of bacteria to survive exposure to host serum, which contains complement proteins capable of lysing pathogens. Many Gram-negative organisms, including *Neisseria gonorrhoeae* and *Escherichia coli*, achieve this through modifications of their outer membrane proteins or the acquisition of protective surface structures (Poirel *et al*., 2018). Serum resistance enhances systemic dissemination and increases the likelihood of bloodstream infections.

2.8.4 Gelatinase Activity

Gelatinase is a specific extracellular proteolytic enzyme that hydrolyzes gelatin, collagen and other protein components of host connective tissues. In Gram-negative bacteria, gelatinase activity enhances virulence by breaking down structural barriers, allowing pathogens to penetrate deeper into host tissues and establish infection. This enzymatic degradation not only facilitates bacterial invasion but also provides essential peptides and amino acids that serve as nutrients, thereby supporting bacterial survival and growth in nutrient-limited host environments (Prestinaci *et al*., 2015).

Additionally, gelatinase activity has been associated with the modulation of host immune responses. By degrading host defense proteins, gelatinase impairs immune recognition and clearance mechanisms, giving bacteria an advantage in evading immune attack. Importantly, studies have also linked gelatinase activity to biofilm development, where the enzyme contributes to the maturation and dispersal of biofilm structures. Biofilm-associated gelatinase production enhances chronic infection potential and increases bacterial tolerance to antibiotics and environmental stressors, making treatment more difficult (Aslam *et al*., 2018).

2.9 Public Health and Environmental Implications

2.9.1 Zoonotic Risks

Rabbits can serve as reservoirs of resistant and virulent Gram-negative bacteria, creating significant zoonotic risks. Bacteria such as *Escherichia coli*, *Klebsiella spp.* and *Salmonella spp.* isolated from rabbits have the potential to be transmitted to humans through direct handling, consumption of contaminated meat or contact with contaminated environments (Massacci *et al*., 2018). The presence of multidrug-resistant strains in rabbits, especially those carrying extended-spectrum β-lactamases (ESBLs), raises the likelihood of treatment failures in human infections.

Such cross-species transmission may contribute to localized outbreaks or foodborne illnesses, particularly in communities where rabbit meat is part of the diet or where rabbits are kept as pets (Pirolo *et al*., 2019). These risks highlight the One Health perspective, where animal, human and environmental health are closely linked.

2.9.2 Environmental Dissemination

The environmental impact of rabbit farming adds another layer of concern in the spread of antimicrobial resistance (AMR). Resistant bacteria and genes shed in rabbit feces can contaminate soil, water and crops, facilitating the horizontal transfer of resistance determinants to other microbial communities (Fasanella *et al*., 2020). Poor waste management practices in rabbit farms, particularly in rural and peri-urban settings, amplify the risk of environmental contamination. This dissemination pathway not only affects local ecosystems but also increases the risk of resistant bacteria entering the wider food chain and community. Moreover, the persistence of resistant organisms in the environment enhances their ability to circulate beyond farm boundaries, potentially reaching wildlife, livestock and ultimately humans. Effective management of animal waste and strict hygienic measures in rabbit farming systems are therefore critical to reducing environmental dissemination and protecting public health.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

This study was carried out at the Laboratory Department of Microbiology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

3.2 Collection of rabbits droppings

With the aid of sterile universal bottles fifteen (15) rabbit stool were collected from three different farm (University of Benin farm, Okene Farm and Animal house). It was immediately taken to the laboratory for bacteriological analysis.

3.3 Dilution Technique

Five (5) sterile test tubes were labeled A, B, C, D and E with the aid of a sterile pipette, 9ml of sterile water was transferred into each test tube. One gram (1g) of the rabbit stool was weighed with electronic scale and transferred into test tube A. It was homogenized and one milliliter (1ml) was pipetted and dispensed into test B and serially diluted to test E. The procedure was repeated for other samples. From the last two test tube (D and E), 1ml was introduced into two sterile petri dishes (duplicate) and already prepared nutrient agar was poured, the Petri dishes were allowed to set and then incubated at 35^0C for 18hrs to 24hrs in an inverted position (Cheesbrough, 2004).

3.4 Identification of the bacteria isolates

Enrichment of the bacteria was done by inoculated into a prepared nutrient broth medium in a sterile test tube. It was then incubated at 37^0C for 18 - 24 hours. The pre-enriched culture was then streaked on the surfaces of selective media (Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar (MSA) agar and incubated for 24hrs at 37^0C (Elanthamil *et al*., 2018).

3.5 Standardization of the Isolates

MacFarland standard (0.5) was prepared by mixing 0.05ml of 1% barium chloride ($BaCl_2$) with 9.95ml of 1% Sulfuric acid(H_2SO_2) to form barium sulphate suspension. The turbid solution (McFarland standard) formed was transferred into a test tube for comparison with different bacterial inoculums suspension (Cheesbrough, 2004)

3.6 Antibiotics susceptibility test

Kirby-bauer disc diffusion technique was used as to determine the antibacterial activity of isolated bacteria. 20ml Mueller Hinton agar plates were prepared following the manufacturer's instructions. 1ml aliquot of each test organism suspension (standardized) was transferred onto the well-dried Mueller Hinton agar plates and was spread evenly following slow rotation of the plates and excess was decanted. The plates were allowed to dry, with the aid of sterile forceps, antibiotic disc was impregnated in the well-dried Mueller Hinton agar plates. The antibiotics disk contains Levofloxacin (20mg), cefuroxime (30mg), Gentamicine (10g), Rifampicin(20mg), Streptomycin (30mg), Azithromycin (10mg) Ciprofloxacin (10mg) Erythromycin (30mg) The plates were incubated for 24h. At 37°C. The resultant visible zones of inhibition were measured in millimetres (mm). Zones were interpreted using the CLSI. (Cheesbrough, 2002).

3.7 Gelatinase production test

The gelatinase production test was carried out by inoculating pure bacterial isolates onto nutrient agar supplemented with 1% gelatin and incubating the plates at 37°C for 72 hours. Following incubation, the presence of a clear zone around the inoculation spot indicated the enzymatic hydrolysis of gelatin, confirming gelatinase activity (Ristow and Welch, 2016).

3.8 Hemolysis

Pure cultures of bacterial isolates were grown on the surface of 5% defibrinated sheep blood agar that is made with nutrient agar and incubated at 37 degree Celsius for 72hrs. Lysing of the red blood cells is indicated by a clear halo around the inoculum spot is indicative of haemolysin production (Ristow and Welch, 2016).

3.9 Serum Resistance Assay

The serum resistance assay was carried out by incubating bacterial isolates in pooled normal human serum (NHS), which served as a source of complement. Test organisms were grown from single colonies in Mueller–Hinton broth. The bacteria were incubated with serum within an interval of 30, 60 and 120 minutes and plated on Mueller–Hinton agar to determine CFU counts. (Necchi *et al*. (2017).

3.10 Capsule Formation test

The capsule staining procedure was carried out using Congo Red as the negative stain. A small drop of Congo Red was placed on a clean glass slide and a loopful of bacterial culture was added aseptically and gently mixed with the stain. The mixture was then spread into a thin smear by dragging another clean slide across the surface at an angle and was allowed to stand for 5–7 minutes. The smear was left to air dry completely without heat fixation to avoid shrinking or destroying the capsules. After drying, the smear was flooded with crystal violet stain for about 1 minute to stain the bacterial cells while leaving the capsules unstained. The excess stain was drained off by tilting the slide at a 45°C angle and counter stained with 20 % copper sulphate solution and the slide was allowed to air dry. Finally, the preparation was examined microscopically under the oil immersion objective (100×), where encapsulated cells appeared as

dark-stained bacterial cells surrounded by clear halos representing the capsules against a darker background (Sofos, J. N. 2009).

CHAPTER FOUR

RESULTS

Table 4.1 shows the total heterotrophic bacterial counts (THBC) of rabbit stool samples obtained from three different farms: University of Benin Farm, Animal House and Okene Farm. The mean bacterial counts varied significantly among the farms, ranging from $2.1 \times 10^6 \pm 1.4$ CFU/g to $12.9 \times 10^6 \pm 1.4$ CFU/g. Specifically, Animal House had the highest bacterial load of $12.9 \times 10^6 \pm 1.4$ CFU/g, followed by Okene Farm with $9.6 \times 10^6 \pm 1.4$ CFU/g while University of Benin Farm had the lowest counts $2.1 \times 10^6 \pm 1.4$ CFU/g. A one-way ANOVA revealed a highly significant difference in the bacterial counts among the three farms ($F(14,15) = 539.90$, $p < 0.001$). Post hoc Bonferroni comparisons further confirmed that the mean bacterial loads of the farms differed significantly from one another ($p < 0.05$).

4.2. Figure 1, shows the distribution of bacterial isolates recovered from rabbit stool samples. A total of six (6) bacteria genera were identified. Among these isolates, *Lactobacillus* sp. was predominant accounting for 26% of the total isolates. Followed by *Neisseria* sp. and *Staphylococcus aureus* with 20% respectively and *Bacillus* sp. and *Escherichia coli* were 13%, while *Salmonella* sp. was the least occurrence with 6%. *Lactobacillus* is a dominant commensal in the gastrointestinal tract of rabbit, where it contributes to intestinal homeostasis, digestion of plant derived carbohydrates and inhibition of pathogenic organisms through lactic acid production.

Table 4.1 Heterotrophic bacteria count of rabbit stools from three different farm.

Uniben Farm	Animal House	Okene Farm	p-Value (0.05)
$2.1 \times 10^6 \pm 1.4$	$12.9 \times 10^6 \pm 1.4$	$9.6 \times 10^6 \pm 1.4$	$p < 0.001$.
$8.9 \times 10^6 \pm 0.7$	$10.2 \times 10^6 \pm 3.5$	$8.9 \times 10^6 \pm 0.7$	$p < 0.001$.
$8.1 \times 10^6 \pm 1.4$	$11.7 \times 10^6 \pm 3.5$	$4.1 \times 10^6 \pm 1.4$	$p < 0.001$.
$4.7 \times 10^6 \pm 0.7$	$7.6 \times 10^6 \pm 2.1$	$4.8 \times 10^6 \pm 0.7$	$p < 0.001$.
$9.6 \times 10^6 \pm 2.2$	$6.9 \times 10^6 \pm 1.4$	$6.1 \times 10^6 \pm 1.4$	$p < 0.001$.

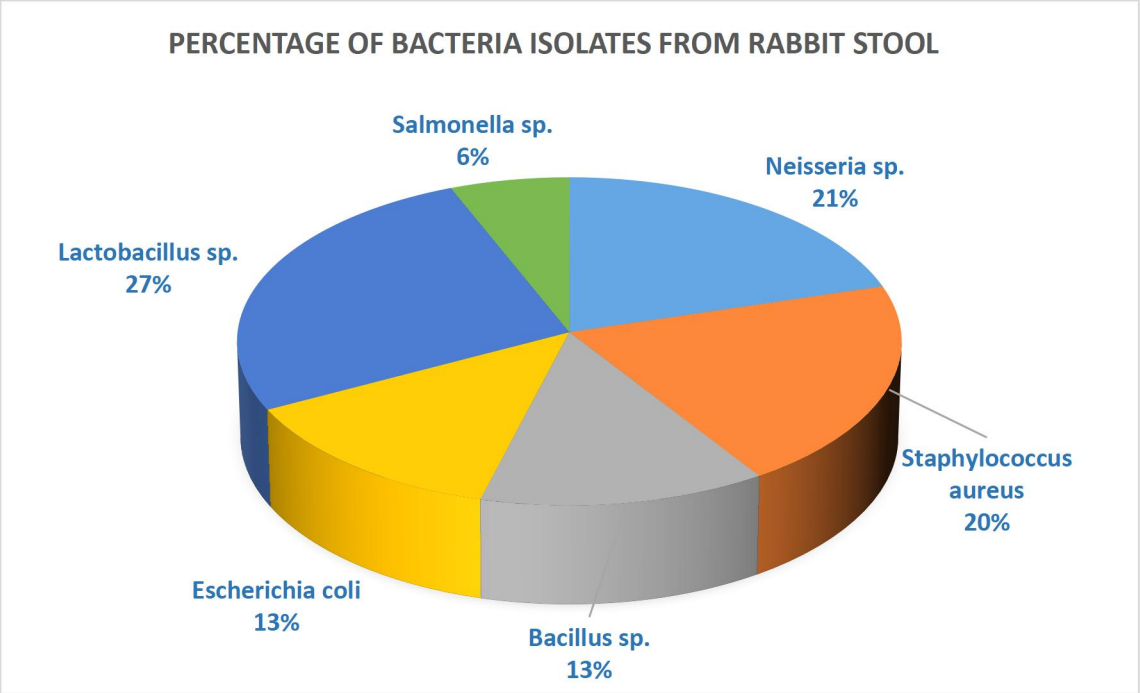

Figure 1:Percentage frequency of bacteria isolates from rabbit stool

Table 4.2 shows the haemolysis activity of bacterial isolates from rabbit stool. *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli*, and *Salmonella* sp. produced 100% β-haemolytic activity, indicating complete haemolysis. The β-haemolysin reflects their capacity to rupture erythrocyte membranes, thereby releasing haemoglobin into the surrounding medium. While *Neisseria* and *Lactobacillus* sp. exhibited no haemolytic activity (γ-haemolysis).

Table 4.3 shows the gelatinase production test of bacterial isolates from rabbit stool. *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. exhibited positive gelatinase activity while *Neisseria* and *Lactobacillus* sp. were negative for gelatinase production with no clear zones around their colonies. The absence of this enzyme in *Lactobacillus* aligns with its nonpathogenic and probiotic nature.

Table 4.4 shows the serum resistance assay of bacterial isolates from rabbit stool. *Neisseria* sp., *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. showed significant resistance to serum killing with viable counts in the range of 10^5 CFU/ml after 120 minutes. While *Bacillus* sp. and *Lactobacillus* sp. were sensitivity to serum activity with decline in viable counts to 10^3 and 10^2 CFU/mL respectively after 120 minutes exposure to serum.

Table 4.5, shows the capsule formation of bacteria isolates from rabbit stool. *Neisseria* sp., *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. expressed a distinct clear halo, indicating the presence of well-defined capsules. While *Lactobacillus* sp. showed no halo. The detection of capsules in *Neisseria*, *S. aureus*, *E. coli* and *Salmonella* sp. is vital as this structural element enable the bacteria to survive in host tissues.

Table 4.2: Haemolysis activity of isolates from rabbit stool

Isolates	n(%)	Type of haemolysin n (α), n (ß), n(γ)
Neisseria sp.	3 (20)	0(0), 0(0), 0(0)
Staphylococcus aureus	3 (20)	6(α), 0(0), 0(0)
Bacillus sp.	2 (13)	0(0), 2(ß), 0(0)
Escherichia coli	2 (13)	0(0), 2(ß), 0(0)
Lactobacillus sp.	4 (26)	0(0), 0(0), 0(0)
Salmonella sp.	1 (6)	0(0), 2(ß), 0(0)

Table 4.3: Gelatinase production test of isolates from rabbit stool

Isolates	n(%)	Gelatinase production	
		n(%) +	n(%) -
Neisseria sp.	3 (20)	0(0)	3(100)
Staphylococcus aureus	3 (20)	3(100)	0(0)
Bacillus sp.	2 (13)	2(100)	0(0)
Escherichia coli	2 (13)	2(100)	0(0)
Lactobacillus sp.	4 (26)	0(0)	4(100)
Salmonella sp.	1 (6)	1(100)	0(0)

Table 4.4: Serum resistance assay of isolates from rabbit stool

Isolates	0 min (T0) CFU/mL	120 min CFU/mL	Interpretation
Neisseria sp.	1.5×10^6	1.5×10^5	Resistant
Staphylococcus aureus	1.1×10^6	1.1×10^5	Resistant
Bacillus sp.	1.0×10^5	1.1×10^3	Sensitive
Escherichia coli	1.2×10^6	1.2×10^5	Resistant
Lactobacillus sp.	1.0×10^5	1.0×10^2	Sensitive
Salmonella sp.	1.0×10^6	1.0×10^5	Resistant

Table 4.5: Capsule formation test of bacteria isolates from rabbit stool

Isolates	**n(%)**
Neisseria sp.	3(100)
Staphylococcus aureus	3(100)
Bacillus sp.	2(100)
Escherichia coli	2(100)
Lactobacillus sp.	0(0)
Salmonella sp.	1(100)

Table 4.6: Antibiotic resistance of bacteria isolates from rabbit stool

Isolates	n (%)	LEV	CN	CEF	RD	CTZ	S	AZM	AMX	CPX	E
Neisseria sp.	3(20)	3(100)	0(0.0)	0(0.0)	3(100)	3(100)	0(0.0)	0(0.0)	0(0.0)	3(100)	0(0.0)
S.aureus	3(20)	3(100)	0(0.0)	0(0.0)	3(100)	3(100)	0(0.0)	0(0.0)	0(0.0)	3(100)	0(0.0)
Bacillus sp.	2(13)	2(100)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)
E. coli	2(13)	2(100)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)
Lactobacillus sp.	4(26)	4(100)	0(0.0)	0(0.0)	4(100)	4(100)	0(0.0)	0(0.0)	0(0.0)	4(100)	0(0.0)
Salmonella sp.	1(6)	1(100)	0(0.0)	0(0.0)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)

KEY: LEV-Levofloxacin, CN-Gentamycin, CEF-Cefuroxime, RD-Rifampicin, CTZ-Ceftazidime, S-Streptomycin, AZM-Azithromycin, AMX-Amoxil, CPX-Ciprofloxacin, E-Erythromycin

0(0.0) – Resistance, (100) – Susceptible

CHAPTER FIVE

DISCUSSION

The total heterotrophic bacterial counts (THBC) of rabbit stool samples from three different locations. Animal House had the highest bacterial load ($12.9 \times 10^6 \pm 1.4$ CFU/g), followed by Okene Farm ($9.6 \times 10^6 \pm 1.4$ CFU/g) and least in University of Benin Farm ($2.1 \times 10^6 \pm 1.4$ CFU/g). A one-way ANOVA revealed a highly significant difference in the bacterial counts among the farms ($F(14,15) = 539.90$, $p < 0.001$) and post hoc Bonferroni analysis confirmed significant variations between all means ($p < 0.05$). This result is in line with the study of Weese, *et* al., (2018), who reported fecal bacterial loads ranging between 10^6 and 10^8 CFU/g in rabbits. Similarly, Siciliano *et al*. (2020), reported bacterial load ranging from $7.5 \times 10^6 – 13.2 \times 10^6$ CFU/g in rabbits stool samples, emphasizing that intensive housing and antibiotic exposure increase bacterial density. The higher count observed in the Animal House and Okene Farm samples could be due to overcrowding, limited ventilation and poor waste management, which create favorable conditions for bacterial proliferation. In contrast, the lower counts observed in UNIBEN Farm may result from better hygiene practices, semi-intensive management and reduced animal-to-animal contact, limiting bacterial transmission. Figure 1 shows that *Lactobacillus* sp. (26%) was the most prevalent among the sixteen (16) isolates studied, followed by *Neisseria* sp. and *Staphylococcus aureus* (20%) respectively and *Bacillus* sp. and *Escherichia coli* (13%) respectively, while *Salmonella* sp. (6%) was the least recovered. This is in line with the study of Velasco-Galilea *et al*., (2018), who reported that *Lactobacillus* and other lactic acid bacteria were among the dominant bacteria in rabbit gastrointestinal microbiota in Spain. However, *Neisseria* sp., *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. were not isolated. The reason for this could be in the difference in the samples analyzed, the

authors focus on the rabbit caecum, while this study focus on the faeces of the rabbit. *Lactobacillus* and other lactic acid bacteria have been shown to form a significant component of rabbit fecal microbial load contributing to intestinal stability and competitive exclusion of pathogens (Velasco-Galilea *et al*., 2018). Similarly, Li *et al*. (2024), isolated *Lactobacillus* sp. from rabbit suffering from bacterial diarrhoea. *Lactobacillus* sp. play a vital role in maintaining gut homeostasis and reducing bacterial diarrhoea in rabbits through immune modulation and restoration of intestinal microbiota balance and a proportion of this bacteria is naturally shed in the faeces as part of the normal intestinal microflora Li *et al*. (2024). However, the relatively high proportions of *Lactobacillus* sp. in the current study could be as a result of the method used in studied culture-based techniques on faecal samples were employed in Nigerian, which differ from the study of Velasco-Galilea *et al*., (2018), in Spain, who employed sequencing techniques. Additionally, husbandry and environmental conditions in Nigeria, such as mixed feed types, lower biosecurity, higher microbial load in bedding and environment may favour opportunistic colonization or overgrowth pathogens.

The predominance of *Lactobacillus* sp. supports its role as a beneficial commensal in rabbits, where it aids in the digestion of plant derived carbohydrates, production of lactic acid and suppression of pathogenic bacteria. The presence of *Neisseria*, *S. aureus*, *Salmonella* and *E. coli* in this study suggested that rabbit gastrointestinal tract can harbor opportunistic pathogens.

The haemolysis activity of bacterial isolates from rabbit stool. *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. produced 100% β-haemolytic activity, indicating complete haemolysis. The β-haemolysin reflects their capability to rupture erythrocyte membranes, thereby releasing haemoglobin into the surrounding medium. This is in consonance with the study of Chai *et al*., (2021), who reported that *Staphylococcus aureus* isolates from

rabbit farms as typically β-haemolytic and harbouring virulence genes in Malaysia. The absence of haemolysis in *Neisseria* sp. and *Lactobacillus* sp. indicated that these isolates likely lack the secreted haemolytic toxins or pore-forming proteins required for erythrocyte lysis. This is in line with the study of Li *et al*. (2024), in *China* who examined the effect of lactic acid bacteria supplementation on rabbit gut health and diarrhoea prevention using molecular microbiome profiling rather than conventional culturing. Additionally, this study is in line with Kolenda *et al*., (2021), who reported that *E. coli* is often haemolytic and carry hly gene associated with enhanced pathogenicity. Furthermore, *Salmonella* sp. strains have been shown to produce pore-forming toxins causing haemolysis on blood agar (Roderer *et al*., 2016). The β-haemolysis in *Bacillus sp.* may be explained by the production of hemolysin BL (Hbl) and other enterotoxins that are linked with haemolytic activity (Ramm *et al*., 2021). the haemolytic capacity observed among these isolates showed the adaptive advantages in colonization and survival in rabbit gastrointestinal tract. β-haemolysins function as virulence determinants that facilitate iron acquisition from host erythrocytes, enhance nutrient availability and promote tissue invasion. Sphingomyelinase C, can disrupt host cell membranes, promoting local inflammation and immune evasion (Chai *et al*., 2021). Similarly, haemolysin (HlyA) enhances bacterial adhesion to intestinal epithelial cells and induces host cytokine responses, thereby contributing to diarrhoeal disease (Kolenda *et al*., 2021).

The gelatinase production test of bacterial isolates from rabbit stool. *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. exhibited positive gelatinase activity while *Neisseria* and *Lactobacillus* sp. were negative for gelatinase production with no clear zones around their colonies. The absence of this enzyme in *Lactobacillus* aligns with its nonpathogenic and probiotic nature. Gelatinase enzyme is an important virulence factor in pathogenic bacteria

like, *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli*, causing tissue degradation and bacterial invasion in the gastrointestinal tracts of rabbit. *Staphylococcus aureus* produces several enzymes and toxins, including gelatinase, which promote inflammation and persistent infections such as enteritis, diarrhea and gut inflammation (Paul *et al*., 2013). Similarly, *E. coli* use gelatinase and other enzymes to break down tissue barriers and invade the intestinal mucosa and causes systemic infections in rabbits (Molino *et al*., 2005). These enzymes make it easier for the bacteria to survive and multiply within the host.

In contrast, *Lactobacillus* sp. lack the genetic element required to produce gelatinase. Unlike pathogenic bacteria such as *Staphylococcus aureus* and *salmonella* sp., which produce gelatinase to degrade host proteins and facilitate tissue invasion. *Lactobacillus* sp. does not possess the gelE gene that encodes this enzyme. Their survival and ecological success depend not on tissue degradation but on carbohydrate fermentation, through which they convert sugars such as glucose and lactose into lactic acid. This fermentative metabolism supports their adaptation to the gastrointestinal tract, where simple carbohydrates and peptides are readily available. In the rabbit gut, the absence of gelatinase production revealed the probiotic and nonpathogenic nature of *Lactobacillus* sp.as this bacterium contributes to gut health rather than tissue damage. *Lactobacillus* help maintain the integrity of the intestinal mucosa while producing lactic acid that lowers gut pH, inhibits harmful pathogens and promotes a balanced microbial community rather than an invasive pathogen (Li *et al*., 2024).

The serum resistance assay of bacterial isolates from rabbit stool. *Neisseria* sp., *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. showed significant resistance to serum killing with a viable count in the range of 10^5 CFU/ml after 120 minutes of exposure to serum. This is in line with the study of Adu-Bobie *et al*. (2020), who reported that these bacterial isolates have the

ability to resist serum killing. While *Bacillus* sp. and *Lactobacillus* sp. were sensitive to serum activity with a decline in viable counts to 10^3 and 10^2 CFU/ml respectively after 120 minutes of exposure to serum. The viable counts at 10^5 CFU/ml after 120 minutes of exposure to serum showed that these isolates possess virulence factors enabling them to survive and multiply in host tissues thereby causing septicemic or enteric disease in rabbits. Serum resistance is an important virulence trait that helps bacteria survive in the bloodstream and spread to other parts of their host. This ability is often linked to surface component such as capsules, lipopolysaccharides and outer membrane proteins, which block the complement system and prevent the formation of the membrane attack complex that would normally destroy bacterial cells (Harrison *et al*., 2023). Similarly, Kolenda *et al*., (2021), reported that Gram-negative bacteria, like *E. coli* and *Salmonella* produce capsular antigens and lipopolysaccharides that inhibit complement activation and phagocytosis as reported in this study. Furthermore, Chai *et al*., (2021), reported that *Staphylococcus aureus* produce protein A, coagulase and clumping factors, which confer protection against opsonization and enhance persistence in host bloodstream.

In contrast, *Bacillus* sp. and *Lactobacillus* sp. were sensitive to serum bactericidal action, with a decline in viable counts to 10^3 and 10^2 CFU/mL respectively after 120 minutes of exposure to serum. The strong reduction in viable count indicated the absence of potent serum-resistance mechanisms, which is typical of non-pathogenic and probiotic. This agrees with the study of Li *et al*. (2024), who reported that *Lactobacillus* sp. is probiotic that lack virulence-associated surface structures and are easily lysed by complement activity.

The capsule formation of bacteria isolates from rabbit stool. *Neisseria* sp., *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli* and *Salmonella* sp. expressed a distinct clear halo, indicating the presence of well-defined capsules. While *Lactobacillus* sp. showed no halo. The

detection of capsules in *Neisseria*, *S. aureus*, *E. coli* and *Salmonella* sp. is vital as this structural element enable the bacterial to survive in host tissues. capsule, composed mainly of polysaccharides and protects pathogenic bacteria from desiccation, phagocytosis and complement-mediated lysis, thereby promoting persistence and invasiveness within host environments (Campos *et al*., 2023). *E. coli*, possess K-antigen capsules which increased serum resistance and colonization ability, enhancing its pathogenic potential (Sarkar *et al*., 2014). Similarly, Tzeng and Stephens, (2021), reported that *Salmonella* sp. and *Neisseria* sp. rely on their capsular polysaccharides to evade host immune responses and establish infection within mucosal surfaces.

In contrast, *Lactobacillus sp.* exhibited no halo, indicating the absence of capsule formation. This finding confirms its nonpathogenic and probiotic nature, as *Lactobacillus* are adapted to symbiotic life within the gastrointestinal tract rather than invasive infection (Lebeer *et al*., 2008). *Lactobacillus* relies on other surface structures such as adhesion proteins, exopolysaccharides and mucin-binding proteins to attach to epithelial surfaces and exert beneficial effects, rather than on virulence-associated capsules (Plaza-Diaz *et al*., 2019). The absence of a capsule supported its role in maintaining gut health, as it allows closer interaction with intestinal epithelial cells and minimizes immune evasion behaviors of pathogens. Within the rabbit gut, *Lactobacillus* contributes to microbial balance by producing lactic acid, lowering intestinal pH and inhibiting the growth of pathogenic bacteria (Flesarova *et al*., 2020).

Tthe antibiotic susceptibility pattern of the bacterial isolates recovered from rabbit. All isolates were completely susceptible to levofloxacin, ciprofloxacin, rifampicin and ceftazidime but exhibited total resistance to gentamicin, cefuroxime, streptomycin, azithromycin, amoxicillin and erythromycin. Bacterial resistance can be attributed to several molecular and biochemical

mechanisms that have been widely reported in both clinical and veterinary isolates. Poirel *et al*. (2018) and Munita and Arias (2016), reported that bacteria employ mechanisms such as enzymatic degradation of antibiotic molecules, alteration of target sites, reduced membrane permeability and active efflux pump expression to survive antimicrobial exposure. β-lactam resistance in *Escherichia coli* is commonly associated with the production of β-lactamases and extended-spectrum β-lactamases (ESBLs), which hydrolyze β-lactam antibiotics such as cefuroxime and amoxicillin, rendering them ineffective. Similarly, macrolide resistance in *Staphylococcus aureus* has been linked to the presence of erm genes, which mediate target site methylation, leading to reduced binding affinity between the drug and bacterial ribosomes (Gao *et al*., 2022).

In addition, *E. coli* also possesses efflux pump systems (AcrAB-TolC) and porin loss mutations, which reduce the intracellular concentration of antibiotics, contributing to multidrug resistance (Li *et al*., 2021). Plasmid-mediated resistance plays a crucial role in the dissemination of resistance determinants among bacteria inhabiting gastrointestinal tract. Yahaya *et al*. (2023) and Savin *et al*. (2021) highlighted that mobile genetic element such as plasmids, transposons and integrons facilitate horizontal gene transfer between commensal and pathogenic bacteria, thereby enhancing the spread of resistance genes across species. This mechanism explains the emergence of multidrug-resistant strains even in farms where some antibiotics are no longer in active use. The persistence of resistance determinants despite changes in antimicrobial practices suggested that environmental and microbial reservoirs maintain these genes, posing continuous threats to both animal and human health.

CONCLUSION

This study revealed that rabbit faecal samples harbor a diverse range of bacterial isolates, including both commensal and potentially pathogenic species. The identification of virulence factors such as haemolysin production, gelatinase activity, capsule formation, and serum resistance among some isolates indicates their ability to cause infections under favourable conditions.The antimicrobial susceptibility testing showed varying resistance patterns among the isolates, with some demonstrating resistance to commonly used antibiotics. This suggests that rabbits may act as reservoirs of antimicrobial-resistant bacteria, which poses a potential risk to public health through direct contact, environmental contamination, or the food chain.Overall, the findings highlight the significance of monitoring bacterial populations in animal sources and reinforce the need for effective control measures to limit the spread of resistant and pathogenic microorganisms.

RECOMMENDATION

i. Improved Hygiene Practices

Proper sanitation should be maintained in rabbit farms and during handling to reduce contamination and transmission of harmful bacteria.

ii. Rational Use of Antibiotics

Antibiotics should be used judiciously in animal husbandry to prevent the development and spread of antimicrobial resistance.

iii. Regular Surveillance

Continuous monitoring of bacterial isolates and their resistance patterns in livestock should be encouraged.

iv. Public Health Awareness

Farmers and handlers should be educated on the risks associated with zoonotic pathogens and the importance of hygiene.

v. Further Research

More studies should be conducted to explore molecular mechanisms of resistance and the transmission pathways between animals and humans.

vi. Policy Implementation

Regulatory bodies should enforce guidelines on antibiotic usage and animal waste management.

CONTRIBUTION TO KNOWLEDGE

This study provides valuable data on bacterial isolates present in rabbit faeces, their virulence characteristics, and antimicrobial resistance patterns. It highlights the role of rabbits as potential reservoirs of pathogenic and resistant bacteria, thereby contributing to public health awareness and supporting efforts toward improved antimicrobial stewardship.

REFERENCES

Adu-Bobie, J., Thomas, K., Amuzu, E., and Lamptey, J. (2020). Serum resistance and virulence of Gram-negative bacteria isolated from rabbits. *Veterinary Microbiology Research, 12*(2), 55–63.

Alvarez, M., García, M. E., Pérez, M., and Blanco, J. E. (2020). *Characterization of Gram-negative bacteria isolated from rabbits: Antimicrobial resistance and virulence potential. Veterinary Microbiology, 243*, 108637

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., and Baloch, Z. (2018). Antibiotic resistance: A rundown of a global crisis. *Infection and Drug Resistance, 11*, 1645–1658

Badagliacca, P., De Lucia, A., Saccares, S., Condoleo, R., Marino, L., and Pepe, M. (2023). *Virulence gene profiles of rabbit enteropathogenic Escherichia coli (REPEC) isolates in rabbit production systems. Veterinaria Italiana, 59*(4), 191–200

Baur, D., Gladstone, B. P., Burkert, F., Carrara, E., Foschi, F., Döbele, S., … Tacconelli, E. (2017). Effect of antibiotic stewardship on the incidence of infection and colonisation with antibiotic-resistant bacteria and *Clostridium difficile* infection: A systematic review and meta-analysis. *The Lancet Infectious Diseases, 17*(9), 990–1001.

Biemer, J. J. (1973). *Antimicrobial susceptibility testing by the Kirby–Bauer disc diffusion method. Annals of Clinical and Laboratory Science, 3*(2), 135–140

Bush, K., and Bradford, P. A. (2019). *Interplay between β-lactamases and new β-lactamase inhibitors. Nature Reviews Microbiology, 17*(5), 295–306

Campos, M. A., Oliveira, A. L., and Silva, R. M. (2023). Capsule-mediated immune evasion in Gram-negative pathogens: Mechanisms and implications. *Frontiers in Cellular and Infection Microbiology, 13*, 1189456.

Chai, L., Yee, K. W., Tan, C. W., and Lim, C. K. (2021). Hemolytic and virulence gene profiles of *Staphylococcus aureus* isolates from rabbit farms in Malaysia. *Veterinary Sciences, 8*(11), 263.

Cheesbrough, M. (2004). *District laboratory practice in tropical countries: Part 2* (2nd ed.). Cambridge University Press.

Clinical and Laboratory Standards Institute. (2020). *Performance standards for antimicrobial susceptibility testing* (30th ed.). CLSI supplement M100. Clinical and Laboratory Standards Institute

da Silva Guedes, J., Velilla-Rodríguez, D., and González-Fandos, E. (2024). *Microbiological quality and safety of fresh rabbit meat with special reference to methicillin-resistant Staphylococcus aureus (MRSA) and ESBL-producing Escherichia coli. Antibiotics, 13*(3), 256

Elanthamil, R., Bandeswaran, C., Tensingh Gnanaraj, P., and Vijayarani, K. (2018). *Isolation and characterization of rabbit gut-transmitted Saccharomyces cerevisiae as a synbiotic*. *International Journal of Current Microbiology and Applied Sciences, 7*(3), 871–882

Fasanella, A., Di Matteo, A., Faleo, S., and Petrella, A. (2020). *Environmental dissemination of antimicrobial resistance in small-scale livestock farming systems. Frontiers in Environmental Science, 8*, 86

Flesarova, S., Sirotek, P., and Tvrznikova, L. (2020). Effects of probiotic *Lactobacillus* strains on intestinal health in rabbits. *Animals, 10*(8), 1301.

Frieri, M., Kumar, K., and Boutin, A. (2017). *Antimicrobial resistance. Journal of Infection and Public Health, 10*(4), 369–378

Gao, Y., Wang, J., and Zhang, X. (2022). *erm* genes and macrolide resistance in *Staphylococcus aureus* from animal sources. *Microbial Drug Resistance, 28*(4), 467–475.

Harrison, E. M., Holmes, M. A., and Holden, M. T. G. (2023). Mechanisms of complement resistance in *Staphylococcus aureus* and Gram-negative bacteria. *Nature Reviews Microbiology, 21*(3), 145–159.

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., … Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet, 387*(10014), 176–187.

Hu, Y., Yang, Q., and Li, J. (2023). Plasmids carrying antimicrobial resistance genes in Gram-negative bacteria. *Microorganisms, 11*(8), 1678

Johnson, T. J., and Nolan, L. K. (2019). *Pathogenomics of the virulence plasmids of extraintestinal pathogenic Escherichia coli. Gut Pathogens, 11*, 10

Jorgensen, J. H., and Ferraro, M. J. (2009). Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases, 49*(11), 1749–1755

Kakkar, M., Chatterjee, P., Chauhan, A. S., Grace, D., Lindahl, J., Beeche, A., … Laxminarayan, R. (2018). Antimicrobial resistance in South East Asia: Time to ask the right questions. *Global Health Action, 11*(1), 1483637.

Kakkar, M., Walia, K., Vong, S., Chatterjee, P., and Sharma, A. (2018). *Antimicrobial resistance in South East Asia: An update. The Lancet Global Health, 6*(3), e283–e284

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., and Laxminarayan, R. (2018). *Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proceedings of the National Academy of Sciences, 115*(15), E3463–E3470

Kolenda, R., Burdukiewicz, M., and Schierack, P. (2021). *Escherichia coli* hemolysin and its role in intestinal diseases. *Toxins, 13*(6), 397.

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Rottingen, J. A., Klugman, K., and Davies, S. (2016). Access to effective antimicrobials: A worldwide challenge. *The Lancet, 387*(10014), 168–175.

Lebeer, S., Vanderleyden, J., and De Keersmaecker, S. C. J. (2008). Genes and molecules of *Lactobacillus* adhesion to the intestinal mucosa. *FEMS Microbiology Reviews, 32*(5), 692–704.

Li, X., Zhang, Y., and Chen, J. (2021). Efflux pumps and porin loss in multidrug-resistant *Escherichia coli* isolates from rabbits. *Antibiotics, 10*(7), 819.

Li, Y., Sun, H., Wang, Z., and Liu, Q. (2024). Characterization of *Lactobacillus* spp. isolated from rabbits with bacterial diarrhea and their probiotic potential. *Frontiers in Veterinary Science, 11*, 1458721.

Liu, X., Yang, Y., Li, Y., Sun, C., and Wang, Z. (2020). *Dissemination of carbapenem-resistant and ESBL-producing Enterobacteriaceae in companion and farm animals: A review. Frontiers in Microbiology, 11*, 565621

Martín-Peláez, S., Moré-Béjar, S., and Pérez, M. (2020). *Use of rabbits as experimental models for studying enteropathogenic Escherichia coli and Salmonella infections. Animals, 10*(12), 2294

Massacci, F. R., Magistrali, C. F., Cucco, L., Curcio, L., Bano, L., Mangili, P., and Scuota, S. (2018). *Characterization of bacterial flora in healthy and diarrheic rabbits with focus on Escherichia coli and Clostridium perfringens. Veterinary Microbiology, 219*, 25–31

Molino, B. F., Gomes, T. A. T., and Campos, L. C. (2005). Gelatinase production and enteric pathogenicity of *Escherichia coli* in rabbits. *Brazilian Journal of Microbiology, 36*(4), 321–326.

Munita, J. M., and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum, 4*(2), 10.1128/microbiolspec.VMBF-0016-2015.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655.

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., ... and Laxminarayan, R. (2022). *Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399*(10325), 629–655

O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Review on Antimicrobial Resistance.

Paul, D., Mandal, S., and Roy, T. (2013). Gelatinase and hemolysin production by *Staphylococcus aureus* isolates and their correlation with enteric infections in rabbits. *Journal of Veterinary Science, 14*(3), 345–352.

Plaza-Díaz, J., Ruiz-Ojeda, F. J., Vilchez-Padial, L. M., and Gil, A. (2019). Evidence of the anti-inflammatory effects of *Lactobacillus* spp. in animal and human models. *Nutrients, 11*(2), 391.

Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., and Nordmann, P. (2018). *Antimicrobial resistance in Escherichia coli. Microbiology Spectrum, 6*(4), ARBA-0026-2017

Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., and Nordmann, P. (2018). Antimicrobial resistance mechanisms in Gram-negative bacteria. *Microbiology Spectrum, 6*(3), 10.1128/microbiolspec.ARBA-0001-2017

Prestinaci, F., Pezzotti, P., and Pantosti, A. (2015). *Antimicrobial resistance: A global multifaceted phenomenon. Pathogens and Global Health, 109*(7), 309–318

Ramm, F., Periasamy, S., and Chandra, G. (2021). Hemolysin BL production and enterotoxins in *Bacillus* species. *Pathogens, 10*(12), 1613

Roderer, D., Glockshuber, R., and Winkler, F. K. (2016). Structural basis of pore formation by *Salmonella* hemolysin. *Scientific Reports, 6*, 33637

Sánchez, S., Martínez, R., Blanco, J. E., and Blanco, J. (2019). *Impact of antibiotic use on intestinal microbiota and antimicrobial resistance in rabbits. Animals, 9*(10), 819.

Sarkar, A., Saha, S., and Chattopadhyay, S. (2014). Capsule polysaccharides and serum resistance in *Escherichia coli*. *Journal of Medical Microbiology, 63*(Pt 3), 342–349

Savin, M., Bierbaum, G., Hammerl, J. A., Heinemann, C., and Klein, G. (2021). Plasmid-mediated antibiotic resistance in animal bacteria: Implications for zoonotic transmission. *Frontiers in Microbiology, 12*, 653595

Segura, P., Martínez-García, R. M., García-García, E., and Pérez-Martínez, G. (2021). *Comparative analysis of gut microbiota composition in humans and rabbits: Implications for translational research. Frontiers in Microbiology, 12*, 653020

Siciliano, G., Cirovic, V., Bonanno, A., Alabiso, M., Mazza, F., Lo Presti, V., and Di Grigoli, A. (2020). Breeding farm, level of feeding, and presence of antibiotics in the feed influence rabbit cecal microbiota. *Animal Microbiome, 2*(1), 14

Skovlund, C. R., Forkman, B., Lund, T. B., Mistry, B. G., Nielsen, S. S., and Sandøe, P. (2023). Perceptions of the rabbit as a low-investment 'starter pet' lead to negative impacts on its welfare: Results of two Danish surveys. *Animal Welfare, 32*,

Sun, C., Wang, Z., Li, Y., and Huang, J. (2024). *Antibiotic resistance spectrums of Escherichia coli and Enterococcus spp. strains against commonly used antimicrobials from commercial meat-rabbit farms in Chengdu City, Southwest China. Frontiers in Veterinary Science, 11*, 1369655.

Sun, J., Li, X., Guo, Q., and Li, S. (2019). *Efflux-mediated antimicrobial resistance in bacteria: Mechanisms, regulation and clinical implications. Frontiers in Microbiology, 10*, 1874

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., … Magrini, N. (2018). Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases, 18*(3), 318–327.

Tzeng, Y. L., and Stephens, D. S. (2021). Capsule in *Neisseria* and *Salmonella*: Structure, function, and vaccine implications. *Frontiers in Immunology, 12*, 688785

Velasco-Galilea, M., Piles, M., Viñas, M., Rafel, O., González-Rodríguez, O., Guivernau, M., Sánchez, J. P., and Lozano, M. (2018). Rabbit microbiota changes throughout the intestinal tract. *PLOS ONE, 13*(9), e0204807.

Weese, J. K., Turner, P. V., and Kylie, J. (2018). Comparison of the fecal microbiota of domestic commercial meat, laboratory, companion, and shelter rabbits (*Oryctolagus cuniculus*). *BMC Veterinary Research, 14*, 143

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., … Thomas, C. M. (2023). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases, 13*(2), 155–165.

Wiegand, I., Hilpert, K., and Hancock, R. E. W. (2008). *Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3*(2), 163–175

World Health Organization Regional Office for Africa. (2024, November 26). *Addressing the challenges of antimicrobial resistance in Africa.* https://www.afro.who.int/countries/nigeria/news/addressing-challenges-antimicrobial-resistance-africa

World Health Organization. (2015). *Global action plan on antimicrobial resistance*. Geneva: WHO.

Yahaya, A., Bello, M., and Mohammed, R. (2023). Horizontal gene transfer and plasmid-mediated resistance in bacteria from animal farms in Nigeria. *Antibiotics, 12*(2), 245

Zhao, L., Yang, S., Liu, J., and Zhang, Y. (2024). *Study on changes in gut microbiota and microbiability in rabbits at different developmental stages. Frontiers in Microbiology, 15*, 1329360

APPENDIX I

Table 7: Cultural characteristics, Morphological characteristics and Biochemical characteristics Of bacterial isolates from rabbit stool

Cultural characteristics				
Colour	cream	Cream	Cream	Cream
Shape	Irregular	Round	Circular	Circular
Elevation	Flat	Raise	Raise	Raise
Margin	Undulate	Smooth	Entire	Entire
Size	Large	medium	medium	Large
Morphological characteristics				
KOH	+ve	-ve	+ve	+ve
Gram stain	-ve	+ve	-ve	-ve
Cell mophology	Rod	Cocci	Cocci	Rod
Cellarrangement	Singly	Cluster	Cluster	Singly
Biochemical characteristics				
Catalase	+	+	-	+
Indole	+	+	+	-
Oxidase	-	-	-	-
Voges-proskauer	Variable	-	-	-
Spore forming		-	-	-
Glucose	+	+	+	+
Lactose	+	+	+	-
Sucrose	+	+	+	-
Mannitol	+	+	+	-
H2S production	-	-	+	+
Identity	*E.coli*	*S. aureus*	*Neisseria* sp	*Salmonella* sp.

APPENDIX II

PREPARATION OF MEDIA

Nutrient Agar

28g of powdered nutrient agar were dissolved in 1000ml of deionized water allowed to soak for 10minutes and then sterilized with an antoclaving for 15minutes at 121^0C allowed to cool and pour into petri dishes.

Eosin Methylene Blue

37.5g of powdered EMB agar was dissolved in 1000ml of deionized water. Allow to soak for 10minute, swirl to mix and sterilize by autoclaving at 121^0C for 15minutes. Allow to cool at 47^0C and pour into petri dishes.

Peptone water / Broth

3.8g of powdered peptone water was dissolved in 280ml of distilled water allow to soak for 10minutes and 5ml was dispensed into 5 labelled sterile test-tubes then sterilized by autoclaving for 15minutes at 121^0C

Thio sulphate citrate Bile Salt (TCBS)

88g of powdered TCBS cholera medium agar was dissolved in 1000ml of deionized water, allow to soak for 10minutes, swirl to mix the bring to the boil and cool to 45^0C and pour into petri dishes

Sammon citrate Agar

24g of powdered SCA was dissolved in 1000ml of deionized water, soak for 10minutes, and swirl to mix. Dispense into test tubes by adding 5ml and sterilized by autoclaving at 121^0C for 15minutes. The medium is set as a slope ensuring that the slant is over a butt about 3cm deep.

Procedure for sub culturing

Pure isolates were obtained by selecting discrete colonies and having them subcultured onto petri dishes containing freshly prepared NA media. The bacteria isolates were also transferred by streak method onto free plates respectively.

MORPHOLOGICAL IDENTIFICATION

Gram Staining

1. A thin smear was prepared on clean glass allowed to air dried and then flame it.
2. The smear was stained with crystal violet for 60 seconds.
3. Rapidly wash off the stain with clean water for 5 seconds.

4. Tip off all the water and cover the smear with lugol's iodine for 60 seconds and washed off under slowly running tap.
5. Decolourized using 90% ehanol and washed immediately with clean water.
6. The smear was covered with safranine reagent for 30 seconds then washed off the stain slowly under running tap.
7. The slide was blot dry using paper towel.
8. The strained cells were examined microscopically with oil immersion using only 100 objective lenses.
9. Gram positive cells stain purple while gram negative cells stain pink or red.

BIOCHEMICAL TEST

Sugar Fermentation (Glucose)

The smear solution was 1% of glucose. The sugar glucose was prepared and sterilized with nutrient agar at 121^0C for 15minutes. Phenol red was used as indicator for acid production. The colony was inoculated on the nutrient agar containing the glucose. The presence of dark color shows the organism can ferment glucose (Cheesbrough, 2004).

Oxidase test

Procedure: place a piece of filter paper in a clean petri dish and add 2 0r 3 drops of freshly prepared oxidase reagent. Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper. Positive colonies turn bluish – purple (Cheesbrough, 2004).

Catalase test

Procedure: 1ml hydrogen peroxide solution was poured in a test tube. A sterile glass rod was used to collect or remove several colonies of the test organism and immersed. In the hydrogen peroxide solution, the test tubes were observed for immediate bubbling of gases which indicate a positive reaction (Cheesbrough, 2004).

Citrate utilization

Procedure: prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8^0C). Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.Incubate at 35^0C for 48hours, look for a bright blue color in the medium (Cheesbrough, 2004).