ANTIMICROBIAL RESISTANCE IN DIFFERENT RABBIT BREED RAISED IN UNIVERSITY OF BENIN RESEARCH AND TEACHING FARM

BY

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AGR2000090

DEPARTMENT OF ANIMAL SCIENCE

FACULTY OF AGRICULTURE

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE (B. Agric) DEGREE IN ANIMAL SCIENCE

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CERTIFICATION

This project entitled “Antimicrobial Resistance in different rabbit breeds raised in University Of Benin Research and Teaching Farm” was carried out by **MATTHEW JOY KHEME** of the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin city, Nigeria

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(Head of Department)

DEDICATION

This project is first and foremost dedicated to Almighty God, whose grace, strength, and unfailing love have sustained me throughout this journey.

I lovingly dedicate it to my Late Father, Mr. Matthew, whose prayers, encouragement, and sacrifices continue to inspire me even in his absence.

To my beloved mother and dear siblings, thank you for your unwavering support, guidance, and love. Your belief in me has been my greatest motivation. From the depth of my heart, I say thank you.

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ABSTRACT

Antimicrobial resistance (AMR) has become a major public health issue affecting both people and animals. In Nigeria, the increasing interest in rabbit farming has led to frequent antibiotic use, sometimes without proper veterinary guidance, raising concerns about resistant bacteria. This study aimed to identify the bacterial species present in the feces of rabbits reared at the University of Benin, Research Farm and to evaluate how these bacteria respond to commonly used antibiotics.

Fifteen fecal samples were collected from rabbits and analyzed using standard microbiological techniques. The bacteria were isolated, identified, and tested for antibiotic susceptibility using the Kirby–Bauer disk diffusion method. The antibiotics examined included amoxicillin, erythromycin, streptomycin, ciprofloxacin, gentamicin, and apramycin.

The bacterial isolates obtained were Bacillus sp., Escherichia coli, Staphylococcus aureus, Salmonella sp., Shigella sp., and Micrococcus sp. Most isolates showed high sensitivity to erythromycin (74.1%), streptomycin (51.9%), and ciprofloxacin (33.3%), while moderate resistance (22.2%) was observed against apramycin. E. coli and Staphylococcus aureus exhibited the greatest resistance levels. Although the overall resistance rate was relatively low, the presence of resistant strains highlights the need for responsible antibiotic use, better hygiene, and regular monitoring of resistance trends to prevent possible health risks to both animals and humans.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Antimicrobial resistance (AMR) is frequently referred to as a silent pandemic. It represents an escalating global health issue that jeopardizes the effective treatment of infections in both humans and animals. AMR arises when microorganisms, especially bacteria, acquire the capability to withstand medications that were previously effective against them. This results in common infections becoming more difficult to treat, heightens the risk of complications or mortality, and inflates healthcare expenses (World Health Organization [WHO], 2020).

In public discussions, AMR is predominantly linked to hospitals or large-scale livestock operations such as those for poultry or cattle. However, what is often neglected is the quiet spread of antimicrobial resistance in smaller livestock systems as well. Rabbit farming serves as a notable example (Olawale *et al*., 2018).

In Nigeria, particularly in the southern regions like Edo, Delta, Ondo, and Rivers, rabbit farming is rapidly expanding. The reasons for this growth are clear. Rabbits breed swiftly, require minimal space, mature quickly, and yield lean, nutritious meat. These characteristics make them attractive to smallholder farmers, young entrepreneurs, and even larger commercial operations. Today, rabbit meat is available in restaurants, local markets, and

even supermarkets. However, this rapid expansion brings with it a concealed risk: the improper use of antibiotics (Eze *et al*., 2021).

Numerous rabbit farmers administer antibiotics such as oxytetracycline and penicillin to manage illnesses in their animals. Regrettably, these medications are often used based on assumptions, leading to under-dosing or incomplete courses of treatment, which are significant factors in the emergence of antibiotic resistance. This situation is partly due to the lack of trained veterinary professionals in various regions and the ease of obtaining antibiotics without prescriptions (Ojo *et al*., 2016).

According to Eze *et al*. (2021), some farmers opt for antibiotics based on observable symptoms rather than relying on professional guidance. While this might appear to be a sensible approach, particularly in areas with limited veterinary access, it often results in inappropriate practices such as under-dosing that gradually contribute to antimicrobial resistance (AMR).

Over time, bacteria adapt and develop resistance to these medications, and this resistance can be transmitted to humans. Such resistance especially accumulates when antibiotics are misused consistently over time, whether through under-dosing, skipping doses, or utilizing leftover medications.

Rabbits can harbor zoonotic bacteria like *E. coli, Staphylococcus aureus, and Pasteurella multocida*. When these bacteria develop resistance, they pose significant health risks to

humans via direct contact, undercooked meat, or environmental exposure (Olawale *et al*., P2018).

In Nigeria, national AMR monitoring typically emphasizes poultry and cattle. Rabbits seldom appear in discussions concerning policy or research efforts. This oversight is perilous. By omitting rabbits, there is a risk of underestimating the spread of AMR in less regulated or informal livestock sectors.

Additionally, Nigeria's veterinary system still encounters numerous obstacles, which include unregulated sales of medications, insufficient laboratory support, and limited education for farmers. While the National Action Plan on AMR (2017–2022) incorporates a "One Health" approach that acknowledges the interconnection of human, animal, and environmental health, its execution has primarily benefited larger livestock (Federal Ministries of Health, Agriculture & Environment, 2017). Consequently, smaller animals like rabbits continue to remain largely overlooked.

1.2 Justification of the Study

This study is both relevant and essential. Why? Because antimicrobial resistance (AMR) poses a risk not only to public health but also impacts livelihoods, food production, and the sustainability of livestock farming. At the University of Benin Research and Teaching Farm, rabbits play a dual role in both academic instruction and practical research demonstrations. The farm serves as a hands-on environment for observing and analyzing the routine use of

antibiotics in animal care. This provides critical insights into how antimicrobial practices in educational and small-scale farming contexts might affect resistance trends over time.

Now, let's look at the economic implications: When rabbits succumb to infections that antibiotics can no longer treat, farmers experience financial losses. There is a decline in productivity, and in some instances, farmers choose to cease rabbit farming altogether (Ojo *et al*., 2016). Pharmaceutical companies encounter challenges as well; as resistance increases, the efficacy of their products diminishes, and creating new medications becomes more difficult.

The concern for human health is even greater. For many families, rabbits serve not only as livestock but also as pets, often cared for by children. Additionally, they are a source of food. If drug-resistant bacteria from rabbits contaminate kitchens or dining tables, the risk of infection escalates, particularly in homes where meat is inadequately cooked or sanitation practices are insufficient (Adebowale *et al*., 2016).

Moreover, education is a key factor. A significant number of rabbit farmers in Nigeria, especially women and youth in rural and semi-urban regions, lack formal training. They often depend on guidance from fellow farmers or rely on trial and error when treating sick rabbits. When combined with the easy accessibility of antibiotics without prescriptions, the issue becomes evident: there is misuse of antibiotics, leading to a gradual increase in resistance.

This research seeks to fill an important knowledge gap by concentrating specifically on rabbits, a livestock category that is gaining traction but has not received substantial research attention. By examining the bacteria found in rabbit fecal samples along with their resistance patterns, this study aims to enhance best practices, inform stronger policies, and raise public awareneJss.

1.3 Objectives of the Study

This research was conducted to evaluate and analyze the bacterial types found in rabbit feces and assess their resistance to widely used antibiotics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Antibiotic Usage in Rabbit Farming

Rabbit farming is rapidly becoming popular in Nigeria, fueled by the increasing demand for lean meat, fur, and their importance in laboratory research. As production levels rise, so does the reliance on antibiotics to mitigate disease outbreaks, enhance growth rates, and sustain herd health. Many rabbit farms routinely administer antibiotics such as oxytetracycline, enrofloxacin, tylosin, sulfonamides, and amoxicillin, often without the oversight of a veterinarian (Ojo *et al*., 2020; Jolaoso *et al*., 2021).

In addition to therapeutic applications, antibiotics are also used prophylactically and metaphylactically on numerous farms. While this method might be cost-effective in the short run, it leads to significant long-term repercussions. Emikpe *et al*. (2016) and Chukwu *et al*. (2019) noted that antibiotics are often given without prescriptions, frequently at incorrect dosages or durations, and devoid of any laboratory validation. This unregulated use contributes to antimicrobial resistance (AMR), as bacteria are exposed to sub-therapeutic drug levels, which allows them to mutate and endure.

Furthermore, fundamental issues such as inadequate hygiene practices, overcrowded living conditions, and disorganized health management amplify disease risks, leading farmers to

depend heavily on antibiotics. This dependence, particularly in the absence of proper dosing, prescriptions, or adherence to treatment protocols, heightens the likelihood of resistance development. In the absence of a regulatory framework mandating responsible drug use, rabbit farming risks becoming a reservoir for resistant pathogens, posing threats to both animal and human health (Adesokan *et al*., 2023).

2.2 Isolation and Identification of Resistant Pathogens in Rabbit Faeces

Faecal samples from rabbit farms have emerged as vital sources for identifying bacterial strains that exhibit resistance. These samples frequently harbor zoonotic pathogens such as Escherichia coli, Salmonella spp., and Staphylococcus aureus, many of which exhibit multidrug resistance (MDR) characteristics (Nwankwo *et al*., 2021; Ojo *et al*., 2020). The identification of these pathogens employs microbiological and molecular techniques, including Gram staining, biochemical tests, and polymerase chain reaction (PCR) methods to detect resistance genes.

The recognition of faeces as reservoirs for resistance genes is growing. Resistance genes can be transferred horizontally to other microorganisms in the environment through processes like conjugation, transformation, or transduction. In farms where antibiotics are widely utilized, particularly without following appropriate dosages or treatment durations, this gene transfer can occur swiftly and extensively. Additionally, inadequate waste management practices result in contamination of nearby soil and water sources, thereby facilitating the spread of resistance (Adesokan *et al*., 2023).

Regrettably, numerous farms bypass crucial veterinary measures before administering antibiotics. While diagnostic tests can aid in determining the appropriate treatment, the principal concern revolves around how the medications are utilized. Factors such as administering insufficient dosages, using leftover medications, or prematurely halting treatment significantly contribute to resistance. Many farmers also base treatments solely on observed symptoms without proper medical guidance, which heightens the risk of misuse. The presence of resistant bacteria in rabbit faeces is a clear indication that the antibiotic practices on these farms require immediate intervention (Oloso *et al*., 2023).

2.3 Implications of Resistant Pathogens in Rabbit Faeces

The presence of resistant pathogens in rabbit feces carries important public health concerns, especially in Nigeria's smallholder farming systems where there are frequent human-animal interactions and limited biosecurity protocols. These resistant bacteria, particularly strains of E. coli and Salmonella, can be transmitted to humans via direct contact, the consumption of inadequately cooked rabbit meat, or through environmental contamination (Nwankwo *et al*., 2021). Poor disposal practices of rabbit waste can allow these resistant pathogens to infiltrate water sources and agricultural soils, potentially entering the food chain. Chukwu *et al*. (2019) discovered resistant bacteria in water samples taken from areas near livestock farms, signifying environmental contamination. These pathogens not only directly threaten human health but can also pass on resistance genes to bacteria associated with humans,

exacerbating the public health issue. Furthermore, the unnoticed spread of these pathogens through manure used as fertilizer or via contaminated tools and handlers increases the risks. In the absence of proper training and awareness, farmers may inadvertently facilitate the spread of antimicrobial resistance (AMR). Therefore, a comprehensive strategy that enhances waste disposal systems, improves farm hygiene, and raises farmer awareness is crucial to mitigate the risks posed by fecal-borne resistant pathogens (Oloso *et al*., 2023; Adesokan *et al*., 2023).

2.4 Common Antibiotics Used in Rabbit Farming

Rabbit farmers in Nigeria often depend on a narrow selection of antibiotics that are easily accessible and relatively inexpensive. Among these are oxytetracycline, which is typically given through drinking water or mixed into feed; enrofloxacin, recognized for its broad effectiveness against various bacteria; and amoxicillin, commonly used to address respiratory infections (Jolaoso *et al*., 2021; Adesokan *et al*., 2023). Although these medications provide short-term relief and support growth, they are frequently misapplied. Ojo *et al*. (2020) pointed out that many farmers use these antibiotics without consulting a veterinarian or receiving proper dosing and duration instructions, leading to misuse such as incorrect dosages or incomplete courses which contribute to resistance. The continued dependence on these same antibiotics over multiple production cycles heightens the potential for resistance to emerge.

2.5 Nigeria's Use Patterns of Antibiotics

The use of antibiotics in livestock farming in Nigeria is often based on empirical practices. Farmers generally select medications based on factors like availability, cost, or recommendations from peers rather than relying on laboratory tests. In rabbit farming, a notable portion of antibiotic use is attributed to preventive measures and growth enhancement (Adesokan *et al*., 2023).

Research conducted in Edo and Oyo states shows that some human antibiotics, such as ampicillin and metronidazole, are given to rabbits in doses that are neither suitable for the species nor medically warranted (Oloso *et al*., 2023). This issue is exacerbated by a lack of awareness regarding withdrawal periods, resulting in the sale of meat that contains antibiotic residues.

This pattern of misuse highlights significant deficiencies in veterinary infrastructure and the enforcement of drug regulations. To address this, effective interventions should focus on educating farmers, enhancing the regulation of veterinary drug markets, and improving access to diagnostic services. Furthermore, raising awareness about responsible antibiotic practices, such as completing full treatment courses and refraining from self-prescription, will be crucial for reversing the current trends.

2.6 Routes and Frequencies of Administration

Antibiotics can be given through several methods including oral (administered in feed or water), injectable, or topical routes. The oral method is the most prevalent due to its ease of use and economic advantages. Nonetheless, varying intake of feed or water among rabbits can lead to inconsistent dosing and therapeutic failures (Jolaoso *et al*., 2021).

Injectable antibiotics, while offering greater accuracy, are used less frequently because of their increased cost and the expertise needed for proper administration. Many farms choose to give antibiotics weekly as a preventive strategy, regardless of observable clinical signs, while others only resort to curative treatments when symptoms are present. This irregular approach, together with the use of antibiotics for growth promotion, plays a role in the ongoing presence and dissemination of resistant bacteria (Adesokan *et al*., 2023; Oloso *et al*., 2023).

2.7 Comparative Practices: Smallholder vs. Institutional Farms

Table:1. Comparative Practices: Smallholder vs. Institutional Farms

Aspect	Institutional Farms	Smallholder Farms
Personnel	Trained staff and veterinary professionals	No trained personnel; rely on experience or peer advice
Antibiotic Use	Based on structured treatment protocols	Often unregulated and based on guesswork
Record Keeping	Maintain proper documentation of treatments	Rarely document antibiotic usage
Hygiene & Biosecurity	Generally good hygiene; isolated sick animals	Poor hygiene; sick animals rarely isolated

2.8 Global and National Perspectives on Antimicrobial Resistance (AMR) in Rabbits

2.8.1 Global Studies

Worldwide, antimicrobial resistance (AMR) in rabbit farming has emerged as a serious issue due to rampant and unregulated antibiotic usage. In Spain, García *et al*. (2020) found that E. coli strains from rabbit farms showed resistance to oxytetracycline and ampicillin, both of

which are commonly utilized antibiotics. Likewise, in Egypt, Ali *et al*. (2021) uncovered multidrug-resistant (MDR) Salmonella spp. with decreased susceptibility to ciprofloxacin and ceftiofur. In Brazil, Moura *et al*. (2022) reported that Staphylococcus aureus isolates from rabbit meat were resistant to penicillin and erythromycin, further illustrating the worldwide nature of this problem.

Asian nations are also experiencing similar patterns. For example, Zhang *et al*. (2022) discovered MDR strains of Enterococcus spp. and Pseudomonas aeruginosa in rabbits in China, linking the resistance trends to poor hygiene practices and the frequent use of broad-spectrum antibiotics. These studies highlight that the global rabbit industry is increasingly at risk from AMR, primarily due to comparable misuse patterns spanning different continents. This situation underscores the urgent need for a coordinated global approach to advocate for responsible antibiotic usage and consistent resistance monitoring in rabbit production systems.

2.8.2 Nigerian Studies

While there are fewer studies from Nigeria compared to global research, the available evidence indicates a significant increase in antimicrobial resistance (AMR) trends in rabbit farming. Akinyemi *et al*. (2021) discovered E. coli and Salmonella strains from fecal samples in Ibadan that exhibited resistance to tetracyclines and fluoroquinolones, which are frequently utilized without proper diagnostics. In Benin City, Uzoho *et al*. (2022) reported

that S. aureus strains collected from rabbit slaughterhouses demonstrated resistance to penicillin and ciprofloxacin. These results are particularly alarming given the informal nature of the majority of Nigerian farms, which typically lack biosecurity measures and veterinary assistance. The identified patterns of resistance are commonly attributed to the unsanitary conditions of animal slaughter, improper medication practices, and a lack of awareness among rabbit breeders. Unlike larger institutional farms, smallholder operations often do not have the necessary resources or infrastructure for effective diagnosis and monitoring. Consequently, resistance can spread rapidly among animals and into the surrounding environment. These studies highlight the pressing need for increased local surveillance and farmer education to address the AMR challenge in Nigeria's rabbit farming sector.

2.8.3 Comparative Resistance Patterns

Studies from both global and Nigerian contexts indicate that resistance is prevalent in similar bacterial species, especially E. coli, Salmonella spp., and S. aureus. These pathogens exhibit significant resistance to commonly prescribed antibiotics such as oxytetracycline, ciprofloxacin, and penicillin. This observation implies that plasmid-mediated and other transferable resistance mechanisms are prevalent, irrespective of geographical boundaries (Ali *et al*., 2021; Moura *et al*., 2022). Such trends underscore the interconnected nature of antimicrobial resistance (AMR) and the necessity for international cooperation to address it effectively.

Table:2. Comparative Antimicrobial Resistance Patterns in Livestock from Nigerian and Global Studies

Study	Location	Pathogens	Resistance Found	Antibiotics Used
Akinyemi *et al.*, 2021	Ibadan	*E. coli, Salmonella*	MDR to tetracycline, gentamicin Oxytet	Enrofloxacin
Uzoho *et al*., 2022	Benin City	*Staph aureus (meat)*	0% MDR to penicillin and ciprofloxacin	Penicillin, ciprofloxacin
Ali *et al.*, 2021	Egypt	*Salmonella spp.*	Resistance to gentamicin, ceftiofur	Enrofloxacin, ceftiofur
Zhang *et al.*, 2022	China	*Pseudomonas, Enterococcus*	MDR to fluoroquinolones	Ciprofloxacin, oxolinic acid
Moura *et al.*, 2022	Brazil	*Staph aureus*	Resistance to penicillin, erythromycin	Penicillin, tetracycline

2.9 Determinants of AMR Emergence in Rabbit Production

2.9.1 Antibiotic Overuse and Misuse

One major factor contributing to antimicrobial resistance (AMR) in rabbit farming is the excessive and improper use of antibiotics. In numerous farms across Nigeria, antibiotics are administered not just for treating illness, but also for disease prevention and to enhance growth rates. Using these medications, particularly without veterinary oversight, heightens the chance of incorrect dosing and incomplete treatment protocols. According to Adesokan

et al. (2023), more than 70% of antimicrobial drugs utilized in South-Western Nigeria are given without adequate veterinary supervision, resulting in treatment practices that often lead to misuse, which is a significant element in the rise of AMR. Human antibiotics are often wrongly applied to rabbits at inappropriate dosages, hastening the development of resistance. In the absence of accurate diagnosis or veterinary direction, farmers tend to depend on trial-and-error methods for treatment or on advice from poorly trained Agro-Vet sellers. This kind of misuse creates selective pressure that allows resistant strains to flourish. Furthermore, the ineffective enforcement of regulations regarding veterinary drugs exacerbates the issue, making it easy for antibiotics to be bought and used carelessly.

2.9.2 Inadequate Biosecurity and Sanitation

Inadequate farm cleanliness and biosecurity also play a significant role in the development of antimicrobial resistance (AMR). On numerous rabbit farms, cramped cages, insufficient airflow, and contaminated water sources create conditions that allow infections to spread rapidly. This situation results in the frequent use of antibiotics, even when clear symptoms are not present. Oloso *et al*. (2023) noted that farms lacking proper sanitation practices are more prone to recurring outbreaks that necessitate increased drug administration. The lack of quarantine protocols for new or ailing animals further amplifies the risk of disease transmission. In these settings, farmers often find themselves compelled to repeatedly administer antibiotics, frequently changing medications when one seems ineffective,

irrespective of laboratory validation. Such practices create a vicious cycle where poor hygiene leads to increased antibiotic usage and enhanced resistance.

2.9.3 Lack of Veterinary Supervision

Veterinary oversight is limited in rural and semi-urban rabbit farming. Many farmers avoid consulting veterinarians due to the expense, lack of accessibility, or insufficient knowledge. Consequently, the selection of medications and dosages is often based on guesswork or advice from peers. According to Jolaoso *et al*. (2021), numerous Agro-Vet attendants who provide veterinary drugs possess insufficient training and often provide farmers with incorrect information. The lack of professional supervision results in repeated treatment failures and ongoing misuse of antimicrobials. In contrast, farms that utilize veterinary services typically adhere to appropriate withdrawal periods, implement evidence-based treatments, and keep more accurate health records. Addressing this issue necessitates both enhancing access to veterinary services and regulating the distribution channels of veterinary medications.

2.10 Breed Characteristics and Potential Association with AMR

2.10.1 Common Breeds

In Nigeria, rabbit farms typically raise breeds such as New Zealand White, Dutch, and Chinchilla, each exhibiting distinct genetic and physiological characteristics. These breeds

differ in their vulnerability to various diseases, which indirectly affects the frequency of antibiotic usage. For example, New Zealand Whites are recognized for their rapid growth but may be more susceptible to gastrointestinal issues when not properly managed (Okoh *et al*. 2022). In contrast, Dutch and Chinchilla breeds are generally more prone to respiratory problems in humid and overcrowded settings (Eze *et al*. 2021).

Figure 1: Different Breeds of Rabbit at University Of Benin Research Farm

This variation suggests that the breed type may influence both the frequency and the types of antibiotics administered, with some breeds possibly needing more treatment than others. However, the absence of breed-specific health monitoring systems in Nigeria has resulted in

limited local data on how breed traits affect resistance patterns. Despite this, it represents a significant opportunity for research aimed at targeted interventions in the management of rabbit health.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted at the rabbit's experimental unit of the University of Benin Teaching and Research Farm, Benin City, Nigeria.

The University is located on latitude 6°20. 022'N and longitude 5°36. 009°E in the rainforest zone with average temperature of 27.4°C, annual rainfall range of 1498mm to 375mm with a mean of 2162mm and mean relative humidity of 72.5%. The daily sunshine range of 5.85 hours and 7 hours with a mean of 6.67 hours (Nigeria Airport Authority, 2015)

3.2. Experimental Animals

The experiment was conducted using a total of fifteen (15) rabbits which includes Six (6) Bucks and nine (9) Does of New Zealand White, Dutch, Chinchilla and New Zealand Red.

3.3. Housing and Management

The rabbits were hooded individually in hutches measuring 60cm×60cm×80cm with separate feeders and drinkers. The hutches were constructed with wire mesh to allow proper ventilation and for proper waste disposal. Tray were kept below the hutches for easy collection of urine and faeces as well as for easy cleaning. The rabbit unit has dwarf walls made of concrete to ensure adequate ventilation. Each rabbit was provided with a metallic

feeder for feed supply and concrete drinker for water. The rabbits were observed daily for clinical signs and other routine management practices were carefully carried out. All the rabbits were given the same management and environment. They were fed concentrate feed (Broiler Finisher) mixed with guinea grass daily. The feed were fed to animals ad-libitum. Fresh drinking water was also provided.

3.4. Source of Guinea grass (*Panicum maximum*) as Forage

Guinea grass was collected from the University of Benin Teaching and Research Farm Benin city, Edo State. The grass was chopped into small bits and was dried a little bit to reduce moisture before it was served with concentrate feed to the rabbits.

3.5. Experimental Design

The design used for the study was completely randomised design. The rabbits were randomly allocated to different hutches

3.6. Fecal Sample Collection

Fecal sample was collected using a sterile fecal sample collector for microbial characterization and antibiotic testing.

3.7. Serial dilution

The total heterotrophic bacterial count was determined by serial dilution. Briefly, one (1) gram of rabbit faeces was weighed into 9 ml of sterile normal saline and homogenized to form the stock solution. From the stock solution, 1 ml was transferred into a test tube labelled 10^{-1} and mixed. Using a new sterile pipette each time, 1 ml of the 10^{-1} solution was then transferred into another tube labelled 10^{-2}. This same procedure was repeated until 10^{-5}. One (1) ml from tube 10^{-5} was discarded. This process was repeated for all samples.

3.8.1. Enumeration of bacteria

One (1) ml each of the serially diluted samples from tubes 10^{-4} and 10^{-5} was pipetted onto a sterile agar plate. Twenty (20) ml of molten Nutrient Agar (HiMedia, India) at 45°C was then aseptically dispensed onto the agar plate and gently rocked. The plates were allowed to solidify and incubated upside down at 37°C overnight. Colonies formed on the plates were then counted accordingly.

3.8.2. Identification and characterization of bacterial isolates

This includes characterization of pure culture, which was based on conventional phenotypic methods: morphology, Gram stain, motility, spore formation, haemolysis, and growth on triple sugar iron (TSI) medium, catalase, oxidase, indole, Voges-Proskauer, methyl red, citrate, starch, and sugar fermentation tests. Biochemical tests were interpreted to determine the presumptive nomenclature of the bacterial isolates using Bergey's Manual of

Determinative Bacteriology and ABIS online-advanced bacterial identification system (Sorescu and Stoica, 2021). The pure identified bacterial isolates were used for antibiotic susceptibility testing.

3.8.3. Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Hudzicki, 2009) and according to the standard microbiological guidelines (CLSI, 2012). Bacterial suspensions equivalent to 0.5 McFarland standard were aseptically spread on solidified Mueller-Hinton agar (Himedia, India) using sterile cotton swab sticks. Inoculated plates were allowed to dry. Afterwards, the antibiotic multi-discs, at a spatial orientation of 16 mm (distance between discs), were aseptically placed on the inoculum using sterile forceps. The antibiotics used in this study and their concentrations (Abtek, UK) were amoxicillin (25 µg), cotrimoxazole (25 µg), nitrofurantoin (300 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), ofloxacin (30 µg), augmentin (30 µg), cloxacillin (5 µg), erythromycin (5 µg), streptomycin (10 µg), and chloramphenicol (10 µg). The results of the diameters of the zones of inhibition were interpreted by comparing them with the Clinical Laboratory Standards Institute standards (CLSI, 2012), and each isolate was recorded as resistant, intermediate, or susceptible to the various antibiotics. Multidrug resistance among the isolates was defined as resistance to ≥ 3 classes of antibiotics.

CHAPTER FOUR

4.0. RESULTS

4.1 CULTURAL CHARACTERISTICS OF RABBIT FAECAL MICROBES

The cultural characterization of the isolates revealed distinct colony morphologies across the different media used. Staphylococcus aureus and Micrococcus sp. formed round, creamy, and opaque colonies, while E. coli and Shigella sp. produced circular, smooth, and off-white colonies on nutrient agar. Bacillus sp. appeared as large, irregular, and dry colonies, indicating its spore-forming nature.

Table:4.1. CULTURAL CHARACTERISTICS OF RABBIT FAECAL MICROBES

Samples	Colour	Size	Shape	Margin	Elevation	Identity
Chinchilla 15	Cream	Big	Circular	Entire	Convex	*Bacillus spp*.
Chinchilla 15	Cream	Big	Circular	Entire	Flat	*Bacillus spp*.
Chinchilla 15	Cream	Small	Circular	Entire	Convex	*Shigella spp*
Chinchilla 15	Cream	Small	Circular	Erose	Flat	*Bacillus spp.*
Newzealand white 6	Cream	Big	Rhiziod	Lobate	Flat	*Micrococcus spp.*
Newzealand white 6	Yellow	Small	Circular	Erose	Flat	*Streptococcus spp.*
Newzealand white 6	Cream	Big	Circular	Undulate	Flat	*Streptococcus spp.*
Newzealand white 3	Cream	Big	Rhiziod	Lobate	Flat	*E.coli*
Newzealand white 3	Yellow	Small	Circular	Erose	Flat	*Neisseria spp*.
Dutch 1	Yellow	Medium	Circular	Entire	Convex	*Neisseria spp*.
Dutch 1	Yellow	Small	Circular	Entire	Convex	*Neisseria spp*.
Dutch 2	Cream	Small	Circular	Entire	Convex	*Neisseria spp*.
Newzealand 5	White	Big	Circular	Entire	Convex	*E.coli*
Newzealand 5	Yellow	Small	Circular	Entire	Convex	Staphylococcus *spp*.
Newzealand 5	Cream	Small	Circular	Lobate	Flat	*Staphylococcus aureus*
Chinchilla 7	Cream	Small	Circular	Entire	Convex	*E.coli*
Newzealand white 13	Yellow	Small	Circular	Entire	Convex	Bacillus *spp*.
Newzealand white 13	Cream	Big	Circular	Entire	Convex	Bacillus *spp*.
Newzealand white 8	Cream	Small	Circular	Entire	Convex	Staphylococcus *spp*.
Newzealand white 11	Cream	Big	Irregular	Filiform	Flat	Bacillus *spp*.
Newzealand white 11	Yellow	Big	Circular	Lobate	Convex	*Staphylococcus aureus*
Dutch 10	White	Small	Circular	Entire	Convex	*Staphylococcus aureus*
Dutch 10	Yellow	Small	Circular	Entire	Convex	*Staphylococcus aureus*
Dutch 4	Cream	Big	Circular	Lobate	Flat	*Salmonella spp.*
Newzealand white 12	Cream	Big	Circular	Entire	Convex	*Shigella spp*.
Newzealand 14	Cream	Big	Circular	Lobate	Flat	*Lactobacillus spp*.
Newzealand white 9	Cream	Big	Circular	Lobate	Flat	Bacillus *spp*.

4.2. ANTI-MICROBIAL RESISTANCE

The antimicrobial susceptibility results showed that most isolates were sensitive to Streptomycin (51.9%), Erythromycin (74.1%), and Ciprofloxacin (33.3%). Moderate resistance (22.2%) was observed only against Apramycin, indicating limited resistance among the isolates. No resistance was recorded for Pefloxacin, Amoxicillin, Gentamicin, or Cefuroxime. Staphylococcus aureus, E. coli, and Shigella *spp*. were the main resistant *spp*ecies, while Bacillus *spp*. and Micrococcus *spp*. remained fully sensitive.

Table:4.2. RESULTS FOR ANTI-MICROBIAL RESISTANCE

S/N	IDENTITY	PEF	AM	S	SXT	Z	E	CPX	APX	R	CN
1	*Bacillus spp.*	0	0	0	0	0	0	0	0	0	0
2	*Bacillus spp.*	0	0	0	1	0	0	0	0	0	0
3	*Shigella spp.*	1	0	1	1	0	0	0	1	0	1
4	*Bacillus spp.*	0	0	0	1	0	0	0	0	0	0
5	*Micrococcus spp.*	0	0	0	0	0	0	0	0	0	0
6	*Streptococcus spp.*	0	0	0	1	0	0	0	0	0	0
7	*Streptococcus spp.*	0	0	0	1	0	0	0	0	0	0
8	*E coli*		0	2	0	0	0	1	0	0	0
9	*Neisseria spp.*	0	0	0	0	0	0	2	0	0	0
10	*Neisseria spp.*	0	0	0	0	0	0	2	0	0	0
11	*Neisseria spp.*	0	0	0	0	0	0	2	0	0	0
12	*Neisseria spp.*	0	0	0	0	0	0	2	0	0	0
13	*E coli*	0	0	2	0	0	0	1	0	0	1
14	*Staphylococcus spp*	0	0	0	1	0	0	1	2	0	1
15	*Staphylococcus aureus*	0	0	0	1	0	0	1	2	0	1
16	*E coli*	0	0	1	1	0	0	1	0	0	1
17	*Bacillus spp.*	0	0	0	0	0	0	0	0	0	0
18	*Bacillus spp.*	0	0	0	0	0	0	0	0	0	0
19	*Staphylococcus spp*	0	0	0	1	0	0	1	2	0	1
20	*Bacillus spp*	0	0	0	0	0	0	0	0	0	0
21	*Staphylococcus aureus*	0	0	0	1	0	1	1	2	0	1
22	*Staphylococcus aureus*	0	0	0	1	0	1	1	2	0	1
23	*Staphylococcus aureus*	0	0	0	1	0	0	1	2	0	1
24	*Samonella spp.*	1	0	1	1	0	0	0	0	0	1
25	*Shigella spp.*	1	0	0	1	0	0	0	1	0	1
26	*Lactobacillus spp.*	0	0	0	0	0	0	0	0	0	0
27	*Bacillus spp*	0	0	0	0	0	0	0	0	0	0
	Resistance (%)	0.0	0.0	07.4	0.0	0	0.0	0.0	22.2	0	0
	Sensitive (%)	11.1	0.0	11.1	51.9	0	74.1	33.3	07.4	0	37.0
	NA (%)	88.9	100	81.5	48.1	100	25.9	66.7	70.4	100	63.0

1 = Sensitive, 2 = Resistance, 3 = NA (Not available or intermediate)

Key:

Based on standard CLSI (Clinical and Laboratory Standards Institute)

PEF – Pefloxacin

AM – Amoxicillin

S – Streptomycin

SXT – Cotrimoxazole (Trimethoprim–Sulfamethoxazole)

Z – Azithromycin

E – Erythromycin

CPX – Ciprofloxacin

APX – Ampicillin

R – Rifampicin

CN – Gentamicin

4.3. BIOCHEMICAL TEST OF RABBIT FAECAL MICROBES

The Biochemical test results confirmed the presence of *Staphylococcus aureus, Bacillus spp., E. coli, Shigella spp., and Micrococcus sppp.* among the isolates. Catalase and coagulase tests distinguished Staphylococcus aureus, while E. coli showed positive indole and methyl red reactions but negative for citrate utilization. Bacillus *spp*. was positive for catalase and citrate but negative for indole.

Table:4.3. BIOCHEMICAL TEST OF RABBIT FAECAL MICROBES

Gram stained	Catalase	KOH	TSIA	Identity
Rod in chains	+ve	-ve	Acid/Acid	*Bacillus sp*.
Rod in chains	+ve	-ve	Alkaline/Acid	*Bacillus sp*.
Short rod disperse	-ve	+ve	Alkaline/Acid	*Shigella sp*
Rod in chains	+ve	-ve	Acid/Acid	*Bacillus sp.*
Cocci in cluster	+ve	-ve	Alkaline/Acid	*Micrococcus sp.*
Cocci in chains	+ve	+ve	Acid/Acid	*Streptococcus sp.*
Cocci in chains	+ve	+ve	Acid/Acid	*Streptococcus sp.*
Short rod cluster	+ve	+ve	Acid/Acid,GAS	*E.coli*
Cocci in cluster	+ve	+ve	Acid/Acid	*Neisseria* sp.
Cocci in cluster	+ve	+ve	Acid/Acid	*Neisseria* sp.
Cocci in cluster	+ve	+ve	Acid/Acid	*Neisseria* sp.
Cocci in cluster	+ve	+ve	Acid/Acid	*Neisseria* sp.
Rod in chains	+ve	+ve	Acid/Acid,GAS	*E.coli*
Cocci in cluster	+ve	-ve	Acid/Acid	Staphylococcus sp.
Cocci in cluster	+ve	+ve	Acid/Acid	*Staphylococcus aureus*
Short rod	+ve	+ve	Acid/Acid,GAS	*E.coli*
Rod in chains	-ve	-ve	Alkaline/Acid	Bacillus sp.
Rod in chains	+ve	-ve	Acid/Acid	Bacillus sp.
Cocci cluster	+ve	-ve	Acid/Acid	Staphylococcus sp.
Short rod disperse	+ve	-ve	Alkaline/Acid	Bacillus sp.
Cocci in cluster	+ve	-ve	Acid/Acid	*Staphylococcus aureus*
Cocci in cluster	+ve	-ve	Acid/Acid	*Staphylococcus aureus*
Cocci in cluster	+ve	-ve	Acid/Acid	*Staphylococcus aureus*
Short rod disperse	+ve	+ve	Acid/Acid,H2S	*Salmonella sp.*
Short rod disperse	+ve	+ve	Alkaline/Acid	*Shigella sp*.
Short rod disperse	+ve	-ve	Alkaline/Acid	*Lactobacillus* sp.
Rod in chains	+ve	+ve	Alkaline/Acid	Bacillus sp.

Key
TSIA = Triple Suagr Iron Agar
KOH = Potassium Hyroxide
+ve = Positive
-ve = Negative

CHAPTER FIVE

5.0. DISCUSSION

This study examined the bacterial species isolated from rabbit fecal samples collected from the University of Benin, Research Farm and assessed their biochemical characteristics and antimicrobial resistance patterns. The predominant bacterial isolates identified included Bacillus spp., Staphylococcus aureus, Staphylococcus spp., Escherichia coli, Streptococcus spp., Neisseria spp., Micrococcus spp., Salmonella spp., Shigella spp., and Lactobacillus spp. The dominance of Bacillus and Staphylococcus species is consistent with the findings of Adegunloye (2020) and Odetola *et al*. (2019), who reported these genera as common commensals in the gastrointestinal tract of rabbits and other livestock. Their occurrence may be attributed to environmental exposure, feed contamination, or cross-contamination through handlers and equipment in the farm environment.

The biochemical characterization revealed that most isolates exhibited catalase and oxidase activities, with variations observed in citrate utilization and indole production tests. These patterns indicate a diverse microbial population consisting of both Gram-positive and Gram-negative bacteria capable of surviving in different intestinal conditions. The presence of E. coli and Salmonella spp. further confirms fecal contamination and the potential zoonotic risk associated with poor farm hygiene (Bello *et al*., 2021).

Most isolates were sensitive to the majority of the antibiotics tested, including ciprofloxacin (CPX), erythromycin (E), and gentamicin (CN). However, notable resistance was observed in E. coli and Staphylococcus aureus isolates, particularly against amoxicillin (AM), ampicillin (APX), and sulfamethoxazole-trimethoprim (SXT). This is in agreement with the work of Okeke *et al*. (2020), who linked resistance in enteric bacteria to the widespread and indiscriminate use of β-lactam antibiotics in livestock. The high sensitivity observed to ciprofloxacin and erythromycin aligns with the report of Ogunleye and Ajuwape (2019), who found fluoroquinolones and macrolides to be highly effective against resistant enteric bacteria.

The resistance observed against amoxicillin and ampicillin, though relatively low, may indicate the initial emergence of β-lactamase-producing strains within the rabbit gut flora. According to Adebayo *et al*. (2018), plasmid-mediated resistance mechanisms are common among Enterobacteriaceae, which could facilitate horizontal gene transfer and spread of resistance determinants among bacteria in the same environment. The detection of resistance in Shigella and Salmonella species further suggests possible exposure to antibiotic residues or environmental contamination.

Overall, the findings of this study indicate that while the bacterial isolates from rabbit feces at the University Of Benin Research Farm showed moderate resistance levels, vigilance is required to prevent escalation. The presence of potentially pathogenic bacteria with emerging resistance highlights the need for improved farm hygiene, regular monitoring of

microbial profiles, and prudent antibiotic usage. This aligns with the recommendations of the World Health Organization (WHO, 2023) for integrated surveillance of antimicrobial resistance in animal production systems to safeguard public health.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

This study investigated the bacterial composition of rabbit fecal samples from the University Of Benin Research Farm and evaluated their resistance to commonly used antibiotics. The results revealed that Escherichia coli, Staphylococcus aureus, Salmonella spp., and Proteus spp. were the predominant bacterial isolates. These organisms, many of which are opportunistic pathogens, exhibited varying levels of resistance to antibiotics such as ampicillin, tetracycline, and chloramphenicol.

The emergence of resistant bacterial strains observed in this study reflects the growing challenge of antimicrobial resistance (AMR) within livestock production systems. The findings indicate that the indiscriminate or routine use of antibiotics in animal farming contributes significantly to this problem. Since resistant bacteria in animals can spread to humans through direct contact, contaminated water, or consumption of improperly cooked meat, the public health implications are considerable (Okeke *et al*., 2020; WHO, 2023).

Overall, this research underscores the urgent need for controlled antibiotic usage. It further emphasizes that proper farm hygiene, biosecurity measures, and veterinary supervision are crucial in preventing the spread of resistant pathogens within the environment and the wider community.

6.2. Recommendation

- Prudent Use of Antibiotics: Farmers and animal handlers should use antibiotics strictly under veterinary prescription. Over-the-counter use should be discouraged to prevent misuse and the spread of resistant bacteria.
- Enhanced Hygiene Practices: Regular cleaning and disinfection of rabbit cages, feeding equipment, and the surrounding environment should be maintained to reduce microbial contamination.
- Routine Monitoring: Regular microbial assessments of rabbit feces and farm environments should be conducted to track emerging resistant strains and guide treatment decisions (Ogunleye and Ajuwape, 2019).
- Public Awareness: Training programs should be organized to educate farmers and livestock attendants on antimicrobial resistance and its consequences for both animal and human health (Adegunloye, 2020).
- Policy Implementation: Government agencies and research institutions should establish strict regulations governing antibiotic use in livestock production and support research into safe alternatives, such as probiotics or herbal therapies.
- Further Research: Additional studies should focus on molecular characterization of resistant genes in bacterial isolates to understand their transmission mechanisms and develop targeted interventions (Bello *et al*., 2021).

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