ISOLATION AND CHARACTERIZATION OF ENTERIC GRAM-NEGATIVE BACTERIA FROM FECAL SAMPLES OF POULTRY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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

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CERTIFICATION

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

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MRS S.O SAHEED DATE

(Project Supervisor)

_____ _____

PROF E.O. IGBINOSA DATE

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, whose grace, guidance and strength made every step of the Journey possible, to my parents for their love, support and prayers; to my siblings for their motivation and encouragement; and finally to myself, for the perseverance and hard work that brought this project to completion.

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ABSTRACT

With the poultry sector being among the fastest growing agro-based industries world-wide, the poultry farms have emerged as a perspective and widely distributed business industry in Nigeria. However the global increase in the incidence of food borne illnesses with poultry and its products being a major contributor to these infections has become a major challenge to poultry farming. The aim of this study is to isolate and identify different enteric bacteria, find the antimicrobial sensitivity profile against the pathogens isolated from selected poultry farms in Benin city, Edo state. A total of fifteen (15) freshly voided fecal sample were collected aseptically from different poultry farms. The samples were cultured on selective and differential media including Eosin Methylene Blue (EMB) agar, MacConkey agar, Salmonella shigella (SS) agar and bacterial isolate were identified based on cultural, morphological and biochemical properties. Antibiotic sensitivity test was carried using Kirby-Buer disk diffusion method. The total heterotrophic bacterial counts obtained from the fecal sample ranged from 1.00 × 106 CFU/g to 65.00 × 106 CFU/g. Five bacterial species were identified: Escherichia coli, Salmonella sp., Klebsiella pneumoniae, Klebsiella oxytoca, and Proteus mirabilis. Among the identified bacterial isolate Escherichia coli had the highest frequency of (53.33%), followed by Klebsiella pneumoniae and Klebsiella oxytoca with a total frequency of (66.67%), Salmonella sp. with a frequency of (26.67%) while Proteus miribilis had the lowest frequency of (4.35%). E. coli and Klebsiella spp. demonstrated resistance to most βlactams, while Salmonella spp. were resistant to β-lactams and gentamicin (aminoglycoside) but showed low susceptibility to fluoroquinolones (ciprofloxacin, ofloxacin). Proteus mirabilis was resistant to all antibiotics tested. Overall, over 80.00% of isolates exhibited resistance to at least two antibiotic classes. These findings underscore the presence of multidrug-resistant enteric bacteria in poultry environments and highlight the need for improved antibiotic stewardship, surveillance, and farm biosecurity.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

The poultry industry is recognized as one of the most rapidly expanding sectors of agriculture globally, driven largely by the increasing demand for poultry meat and eggs as affordable sources of animal protein (Bolan et al., 2019). In agricultural practice, poultry refers to domesticated avian species reared primarily s"poultry" is derived from the French word poul, which traces its origin to the Latin word pullus, meaning young or small animals (Chat et al., 2019).

Nigeria possesses one of the largest poultry populations in Africa, with an estimated 180 million birds, ranking second only to South Africa (SAHEL, 2015). The sector contributes substantially to national food security through the annual production of approximately 650,000 tonnes of eggs and 300,000 tonnes of poultry meat (FAOSTAT, 2017). Furthermore, poultry production serves as an important source of employment, directly engaging over three million individuals while providing a significant proportion of the animal protein consumed by the Nigerian population (Nanchi et al., 2013; Nkukwana, 2018).

Despite its economic importance, poultry farming is frequently associated with microbial contamination arising from the accumulation and improper management of poultry fecal waste. Poultry droppings are widely applied as organic fertilizers in crop production due to their high nutrient content and ability to improve soil fertility and structure (Adekiya et al., 2020; Ajibola et al., 2022). However, the agricultural utilization of untreated poultry manure may facilitate the dissemination of pathogenic microorganisms into the environment, thereby posing significant public health concerns, particularly when contaminated fruits and vegetables are consumed raw or inadequately processed (Chat et al., 2019; Singh et al., 2018).

The burden of foodborne diseases has increased considerably worldwide, with poultry and poultry-derived products serving as important reservoirs of enteric pathogens. Numerous studies have identified Salmonella spp. and Campylobacter spp. as the predominant bacterial agents implicated in poultry-associated foodborne infections in humans (Ifeanyichukwu et al., 2016). Foodborne illnesses contribute substantially to global morbidity, accounting for nearly one billion episodes of acute diarrheal disease annually among children under five years of age, particularly in low- and middle-income regions of Africa, Asia, and Latin America (Mulata et al., 2014). In Nigeria, factors such as inadequate biosecurity measures, poor sanitation practices, and limited access to veterinary healthcare services—especially among small-scale and backyard poultry producers—have been identified as major contributors to the persistence and transmission of these zoonotic pathogens (Adesiyun et al., 2022).

Poultry feed represents another important source of microbial contamination within production systems. Contamination may occur at several points along the feed production chain, including raw material procurement, processing, handling, storage, ingredient mixing, and exposure to contaminated environmental aerosols (Chat et al., 2019). As a result, contaminated feed can act as a vehicle for the introduction and spread of pathogenic microorganisms within poultry flocks, leading to disease outbreaks, reduced productivity, and increased mortality. Among foodborne bacterial pathogens, Salmonella remains one of the most significant, with transmission commonly occurring through the consumption of contaminated poultry meat, eggs, and egg products. Contamination may originate from systemic infection in birds or from fecal contamination during slaughtering, processing, and handling procedures (Abebe et al., 2020; Rahman et al., 2018).

The emergence and dissemination of antimicrobial-resistant bacteria have become major concerns in both veterinary and public health sectors. The extensive use of antibiotics in poultry production has been identified as a key driver of antimicrobial resistance development (Hochmuth et al., 2016). In many production systems, antibiotics are administered not only for therapeutic purposes but also as growth promoters and prophylactic agents to mitigate the effects of poor hygiene and inadequate biosecurity

practices (William et al., 2012). Continuous exposure of bacterial populations to antimicrobial agents exerts selective pressure that favors the survival and proliferation of resistant strains. Consequently, the widespread and often indiscriminate use of antibiotics in poultry farming has contributed to the emergence of multidrug-resistant (MDR) bacteria, thereby compromising treatment efficacy and posing a serious threat to the control and management of infectious diseases in both animal and human populations (Mamza et al., 2010).

1.2 Aims and objectives

This study was therefore designed to isolate and characterize enteric bacteria from poultry farms in Benin city, Edo state.

The specific objective were to;

1. to determine the bacteria load of the samples

2. to isolate and identify enteric bacteria from poultry droppings 3. to carryout antibiotic susceptibility test against the bacteria isolated

4. to determine the multi-drug resistant bacteria isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. Overview of Zoonotic Disease

Zoonotic diseases are infectious conditions caused by bacteria, viruses, fungi, or parasites that are naturally transmissible between animals and humans (Ziaratiet al., 2022). These diseases constitute a major global public health challenge because a substantial proportion of human infections originate from animal sources. It is estimated that approximately 60% of recognized human infectious diseases and nearly 75% of emerging infectious diseases are derived from animal reservoirs, particularly vertebrate species (Bidaisse & Macpherson, 2014). The transmission of zoonotic pathogens may occur through direct contact with infected animals, exposure to contaminated environmental sources, or the consumption of contaminated food and water (Chiebicz et al., 2018).

Among food-producing animals, poultry represents an important reservoir of numerous bacterial zoonotic pathogens of public health significance. Human infections are commonly acquired through the handling, processing, or consumption of contaminated poultry products, particularly meat and eggs (Mead et al., 1999). Several bacterial species associated with poultry have been implicated in foodborne disease outbreaks, including Salmonella spp., Campylobacter jejuni, Listeria monocytogenes, Shigella spp., and pathogenic Escherichia coli strains (Newell et al., 2010). During slaughtering, processing, and distribution, these microorganisms may contaminate carcasses, processing equipment, and surrounding environments,

thereby facilitating their dissemination through the food chain and increasing the likelihood of human exposure (Humphrey et al., 2007).

However, the epidemiology of poultry-associated zoonotic infections extends beyond foodborne transmission. Certain pathogens are transmitted through alternative routes, including inhalation of contaminated aerosols and direct contact with infected birds or their excretions. For example, Chlamydia psittaci, the causative agent of psittacosis, is primarily transmitted through respiratory exposure to contaminated dust particles, feathers, or secretions from infected birds (Harkinezhad et al., 2009). This demonstrates that poultry-associated zoonoses encompass diverse modes of transmission, emphasizing the need for comprehensive surveillance, biosecurity measures, and public health interventions to minimize the risk of pathogen spread from poultry populations to humans.

2.2. Epidemiology of Bacterial Zoonoses in Poultry

Poultry are globally recognized as major reservoirs of zoonotic bacterial pathogens and constitute one of the most important sources of foodborne diseases in humans (Basazinew et al., 2025). These infections are primarily associated with intestinal bacteria that colonize the avian gut and are disseminated through feces, carcass contamination, and cross-contamination during processing and food handling (Ansarifa et al., 2023). The burden of poultry borne zoonoses varies across regions but remains highest in areas where biosecurity, surveillance, and hygiene measures are inadequate (Toloo et al., 2025).

The leading bacterial zoonoses linked to poultry include Salmonella enterica, Campylobacter jejuni, Campylobacter coli, pathogenic Escherichia coli, Listeria monocytogenes, and Clostridium perfringens (Ayoub et al., 2025; Watts et al., 2024). These organisms are responsible for a significant proportion of human gastroenteritis and systemic infections worldwide with both commercial and backyard poultry flocks serving as asymptomatic carriers, highlighting the silent epidemiological role of healthy birds in sustaining transmission cycle (Kelbert et al., 2025).

The foodborne route remains the most dominant mode of transmission (Abou-jaoudeh et al., 2024). Improper slaughtering, unhygienic processing, consumption of undercooked poultry products facilitates bacterial survival and entry into the human population (Cheng et al., 2024). Non-food routes, such as direct contact with live poultry or contaminated environments, also contribute substantially to the infection, particularly among workers in live bird markets, abattoirs, and poultry farms (Elkenany et al., 2025). For instance, recurrent outbreaks of salmonella and campylobacter in the United States and Europe have been linked to backyard poultry handling, underscoring the continued relevance of behavioral and environmental factors in transmission dynamics (Gaonkar et al., 2025).

According to the World Health Organization (WHO) a critical feature of modern poultry borne zoonoses landscape is the emergence of antimicrobial resistance (AMR). Widespread and often unregulated antibiotics use in poultry production has accelerated the development of resistance strains of salmonella, campylobacter, and Escherichia coli, posing major therapeutic challenges in both veterinary and human medicine (Ayoub et al., 2025). Meta-analysis indicates increasing resistance to fluoroquinolones, tetracyclines, and β-lactams among poultry isolates, suggesting selective pressure from antimicrobial misuse in production systems (Basazinew et al., 2025).

The epidemiology of these infections reflects complex interactions between microbial ecology, farm management practices, and human behavior (Tolooe et al., 2025). High income countries have achieved notable decline in certain serotypes following the implementation of integrated control programs, including poultry vaccination and hazard analysis critical control point (HACCP) monitoring (Cheng et al., 2024). However, in many low and middle income regions, weak surveillance, informal poultry markets, and inadequate cold chain systems sustain high infection rates (Abou-jaoudeh et al., 2024). One Health based surveillance integrating human, animals and environmental data has proven valuable for tracing bacterial transmission pathways and evaluating intervention efficiency (Elkenany et al., 2015).

Overall the persistence of bacterial zoonoses in poultry underscores the urgent need for coordinated control strategies encompassing farm biosecurity, hygienic slaughtering, antimicrobial stewardship, and public education on safe poultry handling and cooking (Gaonkar et al., 2025).

2.3. Common Zoonotic Bacteria Found in Poultry

2.3.1. *Salmonella* spp.

Salmonella comprises more than 2,500 identified serotypes (Musa et al., 2017) and is taxonomically divided into two species, Salmonella enterica and Salmonella bongori. Among these, S. enterica is considered the most important from a medical and veterinary perspective and is further categorized into six subspecies (Dhaka et al., 2013; Tegegne et al., 2019). More than 150 serotypes have been implicated in foodborne salmonellosis, with S. Typhimurium and S. Enteritidis recognized as the predominant serovars associated with human infections worldwide (Dhaka et al., 2013; Tadesse & Tessema, 2014; Tegegne et al., 2019). Members of the genus are Gram-negative, facultatively anaerobic, non-spore-forming bacilli belonging to the family Enterobacteriaceae (Chlebicz & Śliżewska, 2018).

Salmonellosis remains a significant global public health concern and continues to be one of the leading causes of foodborne disease in both developed and developing countries, although its prevalence differs considerably across geographical regions (Tadesse & Gebremedhin, 2015). One of the major epidemiological characteristics contributing to its persistence is the occurrence of asymptomatic carrier animals, which facilitate environmental dissemination and maintenance of the pathogen (Kassaye et al., 2015). Salmonella species are ubiquitous in nature and inhabit a broad range of domestic and wild animal hosts (Heredia & García, 2018). Their principal ecological reservoir is the gastrointestinal tract of humans, livestock, birds, reptiles, and insects, from which contamination of food and the environment may occur (Addis & Sisay, 2015).

Human infection is predominantly acquired through the consumption of contaminated food products (Ejo et al., 2016). Foods of animal origin, including meat, eggs, and dairy products, constitute the primary

vehicles of transmission (Kemal et al., 2015). Nevertheless, infection may also result from contaminated drinking water, ingestion of food contaminated with animal feces, or exposure to contaminated food-processing environments and equipment (Dhama et al., 2013).

Non-typhoidal Salmonella (NTS) serovars are particularly associated with animal-derived food commodities such as poultry, eggs, milk, beef, and pork (Musa et al., 2017). Contamination often arises from infected animals entering the food production chain or through cross-contamination during slaughtering and processing operations. The pathogen can spread extensively within processing facilities, increasing the likelihood of contamination of carcasses and edible products (Lee et al., 2015). In eggs, contamination may occur internally through infection of the reproductive tract during egg formation or externally through contact with contaminated environments, especially fecal matter (Taddese et al., 2019). Within poultry production systems, intestinal and fecal contamination of carcasses during processing represents a major route through which Salmonella enters the food chain. In some instances, however, the organism may contaminate food products directly without the involvement of external contamination sources (Tegegne, 2014). Epidemiological evidence indicates that poultry and poultry products account for a substantial proportion of salmonellosis cases, highlighting the public health significance of the poultry industry in disease transmission (Texas Department of Health, 1998).

The incubation period of salmonellosis generally ranges from 12 to 72 hours following exposure (Dhama et al., 2013). Clinical manifestations vary considerably depending on host immunity and pathogen virulence. Disease outcomes may range from mild, self-limiting gastroenteritis to severe systemic illness (Kemal et al., 2015). Common symptoms include diarrhea, abdominal pain, nausea, vomiting, fever, headache, malaise, fatigue, and muscular weakness. The diarrheal episodes may present as watery, greenish, foul-smelling, or occasionally bloody stools containing mucus (Addis & Sisay, 2015). Although most cases resolve without medical intervention, severe complications may occur among young children, older adults, and immunocompromised individuals (Dhama et al., 2013).

Control of salmonellosis requires a multifaceted approach incorporating stringent biosecurity measures, effective contamination control programs, and improved food processing and storage practices. Proper

food hygiene, adequate cooking and reheating, pasteurization of milk, and maintenance of appropriate refrigeration temperatures are essential preventive strategies (Addis & Sisay, 2015). Furthermore, the integration of attenuated recombinant live Salmonella vaccines into comprehensive control programs targeting animals, feed, and food products has demonstrated potential for reducing the burden of salmonellosis in both human and animal populations (Kemal et al., 2015).

2.3.2 Escherichia coli

Escherichia coli is a Gram-negative, rod-shaped member of the family Enterobacteriaceae. The bacterium is generally motile due to the presence of peritrichous flagella and possesses fimbriae that enhance adherence to host tissues. It is also capable of fermenting glucose and a variety of other carbohydrates (Addis & Sisay, 2015). Most strains exist as harmless commensals within the lower intestinal tract of humans and animals, where they contribute to digestive processes and maintenance of microbial homeostasis (Abreham et al., 2019; Disassa et al., 2017; Taye et al., 2013). Nevertheless, certain strains have acquired specialized virulence factors that enable them to cause disease in both humans and animals (Bekele et al., 2014). These pathogenic strains are of considerable zoonotic importance because they can be transmitted between animal and human populations through food and environmental exposure (Messele et al., 2018). Among them, Shiga toxin-producing E. coli (STEC) are particularly noteworthy due to their involvement in severe foodborne outbreaks and their potential to cause life-threatening disease (Elmonir et al., 2018).

Among pathogenic serotypes, E. coli O157:H7 is one of the most extensively studied because of its strong association with foodborne disease outbreaks (Assefa & Bihon, 2018; Bedasa et al., 2018; Bekele et al., 2014; Mustafa & İnanç, 2018). This serotype belongs to the STEC group and represents a major zoonotic pathogen of global concern (Assefa, 2019; Haile et al., 2017; Taye et al., 2013). Outbreaks linked to E. coli O157:H7 have been reported across North America, Europe, Asia, Australia, and several African countries (Abreham et al., 2019; Disassa et al., 2017). In the United States, it has been estimated to cause approximately 74,000 infections and over 60 deaths annually (Bedasa et al., 2018).

Transmission of E. coli O157:H7 occurs primarily through ingestion of contaminated food or water, although direct person-to-person spread and occupational exposure have also been documented (Bekele et al., 2014). As a foodborne zoonotic pathogen, E. coli is most frequently associated with contaminated foods of animal origin, which serve as important vehicles for human infection (Messele et al., 2018).

The incubation period generally ranges from two to ten days. Clinical manifestations commonly include diarrhea, abdominal pain, and vomiting. In severe cases, infection may progress to hemorrhagic colitis characterized by bloody diarrhea. Serious complications such as hemolytic uremic syndrome (HUS), which can result in acute kidney injury, and thrombotic thrombocytopenic purpura (TTP) may also develop (Dhama et al., 2013; Mersha et al., 2010).

Avian pathogenic E. coli (APEC) is predominantly associated with extraintestinal diseases in poultry, including colibacillosis, pericarditis, and airsacculitis, all of which contribute substantially to economic losses within the poultry industry (Ewers et al., 2022). Although traditionally regarded as an avian pathogen, increasing evidence suggests that APEC possesses zoonotic potential. Comparative studies have demonstrated that APEC and human ExPEC strains share several important virulence-associated genes, including iss, iutA, hlyF, ompT, and iroN, which enhance bacterial survival, iron acquisition, and systemic dissemination (Ewers et al., 2022; Johnson & Nolan, 2023). Furthermore, both groups frequently belong to phylogenetic clusters B2 and D and often harbor ColV virulence plasmids, indicating a close genetic relationship (Mora et al., 2024).

Molecular epidemiological investigations have reinforced this association by demonstrating the presence of APEC-like strains in retail poultry products and their capacity to establish infections in mammalian experimental models, suggesting that poultry meat may serve as a potential transmission vehicle to humans (Manges et al., 2019). However, despite the considerable overlap in virulence characteristics between APEC and human ExPEC strains, definitive evidence demonstrating that APEC independently causes disease in humans under natural conditions remains limited (Kafle et al., 2023). Consequently, APEC is currently regarded as a potential zoonotic pathogen and an important reservoir of virulence

determinants and antimicrobial resistance genes that may be transferred to human-associated E. coli populations through foodborne or environmental pathways (Ewers et al., 2022; Kafle et al., 2023).

Prevention of foodborne illnesses caused by E. coli relies on adherence to the same fundamental principles used to control other bacterial foodborne diseases, including proper food handling, adequate cooking, good personal hygiene, prevention of cross-contamination, and consumption of safe water and properly processed food products.

2.3.3 Other Gram-Negative Bacteria Isolated from Poultry

Beyond established zoonotic pathogens such as Salmonella, Campylobacter, and Escherichia coli, a range of additional Gram-negative bacteria are commonly recovered from poultry and their surrounding environments. These include Proteus spp., Gallibacterium anatis, Pasteurella multocida, Pseudomonas aeruginosa, and Klebsiella spp., most of which are regarded as opportunistic organisms with variable public health relevance (Olowe et al., 2022; Christensen et al., 2021).

Proteus species are frequently isolated from contaminated litter, fecal material, and feed sources within poultry production systems. In avian hosts, they have been implicated in septicemic conditions and egg contamination, while in humans they are mainly associated with opportunistic infections such as urinary tract infections and wound sepsis under predisposing conditions (Olowe et al., 2022).

Gallibacterium anatis, previously grouped under the genus Pasteurella, is increasingly recognized as an emerging avian pathogen. It primarily targets the reproductive and respiratory systems of chickens, leading to disorders such as oophoritis and reduced egg yield. Despite its pathogenicity in poultry, current evidence indicates that it remains largely host-restricted, with no confirmed association with human infection (Christensen et al., 2021).

In contrast, Pasteurella multocida, the etiological agent of fowl cholera, has a clearer zoonotic relevance. Although primarily an animal pathogen, it can infect humans following bites, scratches, or occupational exposure, where it typically causes localized soft tissue, wound, or respiratory tract infections (Wilson et al., 2023).

Pseudomonas aeruginosa is an environmental opportunist capable of surviving in diverse ecological niches, including poultry production systems. In birds, it has been linked to septicemia and skin infections, while in humans it is particularly problematic in immunocompromised individuals due to its capacity to cause severe opportunistic infections (Adetunji et al., 2021).

Similarly, members of the genus Klebsiella, especially K. pneumoniae, have been isolated from avian respiratory tissues and reproductive structures such as yolk sacs. Some strains share genetic determinants of virulence and antimicrobial resistance with human clinical isolates, indicating a limited but notable zoonotic and epidemiological overlap (Ferdous et al., 2020).

Although most of these organisms are not primary zoonotic pathogens, their significance lies in their ability to act as reservoirs of virulence factors and antimicrobial resistance genes. These genetic elements are often located on mobile genetic platforms such as plasmids and integrons, which facilitate horizontal gene transfer to other bacterial populations, including established human pathogens (Kafle et al., 2023)..

2.4. Factors Contributing to the Presence and Transmission of zoonotic Bacteria in poultry

The maintenance and spread of zoonotic bacteria within poultry production environments are driven by a complex interaction of ecological, managerial, and microbiological factors. The microbial ecology of poultry is shaped by host–environment interactions, where diverse intestinal microbial communities coexist with pathogenic organisms. This dynamic can favor colonization and persistence of foodborne pathogens such as Campylobacter, Salmonella, and Listeria monocytogenes, particularly when the gut microbiota is disrupted by external pressures (Kers et al., 2018). Key determinants of gut microbial balance include feed composition, antimicrobial exposure, housing conditions, and environmental stress, all of which influence both host resistance and the level of bacterial shedding into the environment.

Suboptimal husbandry practices play a central role in amplifying pathogen transmission within poultry farms. High stocking density, poor litter quality, contaminated feed and water sources, and inadequate waste management collectively create conditions that support bacterial survival and facilitate rapid spread between birds (Sibanda et al., 2018). In addition, insufficient cleaning and disinfection between production cycles allow residual contamination to persist within housing facilities, increasing the risk of both horizontal transmission within flocks and carryover infection to subsequent production batches. Biosecurity weaknesses, including unrestricted human access, shared farming equipment, and inadequate control of mechanical vectors such as rodents and insects, further enhance the dissemination of pathogens across production units (Akter et al., 2025).

Critical stages of poultry production and processing also contribute substantially to microbial contamination. During handling activities such as catching, crating, and transportation, birds experience stress that often leads to increased defecation and subsequent bacterial shedding. When combined with poorly sanitized transport containers and vehicles, these conditions promote cross-contamination between flocks (Dos Santos et al., 2024). Similarly, at the slaughter and processing level, deficiencies in scalding, evisceration, and carcass dressing practices can result in intestinal rupture and direct contamination of meat surfaces with enteric pathogens (Girma, 2015).

At a systemic level, inadequate surveillance systems and weak enforcement of food safety regulations further contribute to the persistence of zoonotic bacteria along the poultry production chain, thereby sustaining a continuous public health risk (Grace, 2024). Consequently, effective control requires strict adherence to biosecurity principles, improved hygiene practices across all production stages, continuous monitoring of feed and water quality, and enforcement of sanitary standards in processing facilities.

Compounding these challenges is the widespread and often unregulated use of antibiotics in poultry production, which has facilitated the emergence and dissemination of antimicrobial-resistant strains of Salmonella, Campylobacter, and Escherichia coli. These resistant organisms are frequently excreted in

feces, contaminating the surrounding environment and serving as reservoirs for resistance genes. Through horizontal gene transfer mechanisms, these resistance determinants can spread to other bacterial populations, thereby complicating treatment outcomes in both veterinary and human medicine and posing a significant global health concern (Ayoub et al., 2025).

2.6. Previous Research Studies in Nigeria

A study conducted by Aboaba and Smith (2005) investigated the occurrence of Campylobacter species in poultry farms across the Lagos area of Nigeria. Sampling fecal droppings from 50 farms, the researchers employed selective enrichment and culture techniques for the isolation of thermophilic Campylobacter spp. A total of 15 isolates were recovered, with 66.7% identified as C. coli and 20.0% as C. jejuni, while the remaining isolates were distributed among other biotypes. This early study demonstrated that poultry farms in Nigeria serve as important reservoirs for zoonotic Campylobacter spp., highlighting their epidemiological significance in local production systems.

More recently, Adetunji et al. (2025) examined the genomic diversity and antimicrobial resistance profiles of Escherichia coli and Salmonella isolates from commercial poultry farms in Oyo State, Nigeria. The study, which covered 25 farms, reported a 48.0% occurrence of Salmonella, including multiple serovars such as S. Kentucky ST198, alongside a 52.0% prevalence of ESBL-producing E. coli. Furthermore, approximately 70.0% of the Salmonella isolates demonstrated resistance to fluoroquinolones. These findings underscore the substantial public health risk posed by multidrug-resistant zoonotic bacteria circulating within Nigerian poultry production systems.

2.7. Strategies for mitigating zoonotic bacterial transmission in poultry

Effective control of zoonotic bacterial transmission from poultry to humans requires an integrated, multi-sectoral approach encompassing biosecurity enhancement, good agricultural

practices, antimicrobial stewardship, vaccination, and food safety interventions across the entire production continuum. Intervention strategies must be implemented from hatchery to retail stages to disrupt transmission pathways and reduce the burden of zoonotic pathogens such as Salmonella, Campylobacter, and Listeria monocytogenes (FAO & WHO, 2019).

2.7.1 Biosecurity and farm hygiene

Robust biosecurity measures constitute the primary defense against introduction and dissemination of zoonotic bacteria in poultry systems. Prevention of pathogen ingress through controlled farm access, sanitation protocols, and all-in/all-out production systems significantly reduces microbial colonization pressure (Newell et al., 2011). Practices such as restricted entry points, footbath disinfection, and routine cleaning and decontamination of equipment are essential in limiting environmental contamination. Empirical evidence indicates that enhanced hygiene management and vector (e.g., rodent and insect) control can markedly reduce Campylobacter colonization in poultry flocks (Jørgensen et al., 2011).

2.7.2 Probiotics, prebiotics, and competitive exclusion

Biological control strategies such as probiotics, prebiotics, and competitive exclusion products offer sustainable alternatives to antimicrobial use by modulating gut microbiota composition. Competitive exclusion cultures composed of non-pathogenic intestinal microflora inhibit colonization by pathogens such as Salmonella through competitive nutrient utilization and receptor occupancy within the gastrointestinal tract (Cox & Pavic, 2010). Probiotic strains, including Lactobacillus spp. and Bifidobacterium spp., have been demonstrated to reduce

intestinal loads of Salmonella and Campylobacter in broilers while simultaneously enhancing mucosal immunity and host defense mechanisms (Kogut & Arsenault, 2016).

2.7.3 Vaccination

Vaccination represents a critical preventive intervention, particularly against Salmonella enterica infections in poultry. Both live attenuated and subunit vaccines have been shown to reduce bacterial colonization and fecal shedding, thereby decreasing transmission within flocks (Desin et al., 2013). Immunization of breeder and layer birds is especially important, as it reduces vertical transmission and egg contamination risks. Although effective vaccines against Campylobacter are still under development, experimental studies targeting flagellar proteins and adhesins have demonstrated promising immunogenic potential (Meunier et al., 2017).

2.7..4 Antimicrobial stewardship

Antimicrobial stewardship programs are essential for curbing the emergence and spread of resistance in poultry systems. Rational antibiotic use—characterized by reduced reliance on prophylactic and growth-promoting applications, coupled with targeted therapy guided by susceptibility testing—helps preserve antimicrobial efficacy (Landers et al., 2012). Countries that have implemented structured stewardship frameworks, such as Denmark and the Netherlands, have reported substantial reductions in antibiotic consumption without compromising production efficiency (Van Boeckel et al., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Benin City, the capital of Edo State, located in the South-South geopolitical zone of Nigeria. Benin City is a prominent urban center known for its dense population, modern infrastructure, and a rising number of poultry ownership. The city lies between latitude 6.34°N and longitude 5.62°E and experiences a tropical climate with alternating wet and dry seasons, which creates favorable environmental conditions for microbial survival and transmission. Sampling location includes residential areas within Ugbowo (Osasogie and Ekosodin), New Benin, Uselu market, where commercial and household poultry farms are common.

3.2 Study Population

The study population consisted of poultry birds especially chicken (*Gallus gallus domesticus*) kept as a source of meat, eggs, and for sale in Benin City. Samples were collected from both male and female chickens across various ages.

3.3 Sample Collection

A total of fifteen (15) freshly voided fecal samples were aseptically collected from random poultry farms. Samples were obtained directly from the ground immediately after defecation to avoid environmental contamination. Using sterile spatulas, approximately 5 grams of fecal matter

were transferred into sterile, wide-mouthed screw-capped universal bottles labeled with date, sex, age, and location. The samples were immediately transported to the microbiology laboratory at the University of Benin for analysis within two hours. Samples not processed immediately were refrigerated at 4°C and analyzed within 6 hours to preserve microbial viability.

3.4 Sterilization of Materials

All glassware such as Petri dishes, conical flasks, McCartney bottles, and pipettes were thoroughly washed with detergent, rinsed in distilled water, air-dried, and wrapped in aluminum foil. These were sterilized in a hot-air oven at 160°C for one hour. Culture media and other heat-sensitive materials were autoclaved at 121°C for 15 minutes under 15 psi pressure. Aseptic procedures were strictly followed, including the use of Bunsen burner flames to maintain sterile zones and routine disinfection of work surfaces using 70% ethanol before and after handling samples..

To maintain sterility and minimize contamination, all reusable glassware—including McCartney bottles, Petri dishes, test tubes, conical flasks, measuring cylinders, and pipettes—was sterilized in a hot-air oven at 160°C for 1 hour before use. Similarly, microbiological media such as Nutrient Agar, MacConkey Agar, Citrate Agar, Salmonella–Shigella (SS) Agar, Eosin Methylene Blue (EMB) Agar, and Mueller–Hinton Agar were sterilized by autoclaving at 121°C for 15 minutes, following standard microbiological protocols. Freshly prepared agar media, agar slants, and biochemical reagents were subsequently stored under refrigeration at 3–4°C to preserve their quality and performance. Throughout the inoculation and subculturing procedures, strict aseptic techniques were consistently observed to prevent cross-contamination and ensure the reliability of microbiological findings.

3.5. Enumeration and Isolation of Bacterial Isolates

Enumeration of bacteria colonies was carried out by serial dilution method. An aliquot of 1 mL of the fecal suspension was transferred into a sterile test tube containing 9 mL of sterile normal saline to create a 10^1 dilution. The mixture was vortexed to ensure uniform distribution of microbial cells. This process was repeated sequentially to prepare higher dilutions, including 10^2, 10^3, and 10^4, using fresh sterile saline for each dilution step. An appropriate aliquot (0.1 mL) from each dilution was aseptically plated onto Nutrient Agar, MacConkey Agar, and Salmonella-Shigella Agar using a sterile wire loop, as described by Cheesbrough (2002). The inoculated plates were incubated aerobically at 37°C for 24 to 48 hours.

After incubation, bacterial growth was observed and colony-forming units (CFU) were counted from plates with countable colonies (typically 30–300 colonies per plate) using the formular

$$\text{CFU/g} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{volume plated (mL)}}$$

The bacterial load was then calculated and expressed in CFU/g of feces. Counts exceeding **10^5 CFU/g** were considered significant, suggesting the potential for zoonotic transmission and active colonization. Pure colonies were further sub-cultured onto fresh MacConkey Agar plates to obtain isolated pure strains. These isolates were preserved on Nutrient Agar slants and stored at **4°C** for subsequent identification.

3.6 Bacterial Identification

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial isolates.

3.6.1 Gram staining

Smears of the bacterial isolates were first prepared on clean, grease-free glass slides and subsequently heat-fixed to ensure firm adhesion of the cells and preservation of cellular morphology. The fixed smears were initially stained with crystal violet for one minute, serving as the primary stain, after which excess stain was gently rinsed off using distilled water.

Following this, Gram's iodine solution was applied as a mordant and allowed to act for one minute to facilitate the formation of a crystal violet–iodine complex within the bacterial cells. The slides were then washed again with distilled water and decolorized using 95% ethanol for approximately 30 seconds, followed by a further rinse with distilled water to halt the decolorization process.

Thereafter, a counterstain of safranin was applied for one minute to provide contrast by staining decolorized cells. The slides were finally washed, air-dried, and examined under a microscope using the oil immersion objective lens for detailed morphological observation and Gram reaction differentiation (Fawole & Oso, 2004).

3.6.2. Biochemical Test

3.6.2.1. Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates

smeared on a slide. The production of gas bubble indicated catalase enzyme positive (Cheesbrough, 2006).

$$H_2O_2 \rightarrow 2\ H_2O + O_2$$

3.6.2.2. Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test (Cheesbrough, 2006).

$$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

3.6.2.3. Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result(Cheesbrough, 2006).

3.6.2.4. Indole Test

The indole test is a biochemical assay used to determine the ability of a microorganism to metabolize the amino acid tryptophan through the activity of the enzyme tryptophanase, resulting in the production of indole. This test is particularly useful in the differentiation and identification of members of the family Enterobacteriaceae and other enteric bacteria.

The procedure involves the use of Kovac's reagent, which contains hydrochloric acid, p-dimethylaminobenzaldehyde, and amyl alcohol. The test organism is inoculated into a suitable tryptophan-containing broth medium and incubated at 37°C for approximately 18 hours to allow bacterial growth and enzymatic activity. Following incubation, 5 mL of Kovac's reagent is carefully added along the inner wall of the culture tube. The formation of a distinct bright red ring at the interface between the reagent layer and the broth within a few seconds indicates the presence of indole, signifying a positive reaction. Conversely, the absence of red colour development indicates that indole was not produced and the organism is considered indole-negative (Cheesbrough, 2006).

3.6.2.6. Urease test

The test was used to determine the ability of microorganisms to degrade urea by means of the enzyme urease. The medium was carefully prepared according to the manufacturer's instruction, dispensed into tubes in slant form and the test organisms inoculated by streaking the slant surface and stabbing the butt of the tube with a sterile wire loop and straight wire respectively. After incubation for 24hrs, a positive result for motility was noted by the organism's growth deviation from its line of inoculation (haziness in the tube). Non-motile organisms generally grew within their stab line (line of inoculation) leaving the surrounding medium clear. Urease positive organisms turned the media bright red-blue due to the hydrolysis of urea in the presence of the indicator phenol red (Olutiola *et al*., 2000).

2.7 Antibiotic susceptibility test

The identified bacterial colonies were subjected to antibiotic susceptibility testing (AST) to determine their resistance or susceptibility to commonly used antibiotics in the locality. The test was conducted using the disc diffusion method, with standard antibiotic discs manufactured by

Oxoid, UK. The specific antibiotics tested included Sparfloxacin, Ciproflaxacin, Amoxicillin, Augmentin, Pefloxacin, Gentamycin, Tarivid**.** For the assay, bacterial cultures grown for 18–24 hours were standardized to 1.50×10^8 cells/mL according to the McFarland standard. Using a sterile loop, the bacterial inoculum was evenly streaked onto Mueller-Hinton Agar (MHA) plates. The antibiotic discs were carefully placed on the plates using sterile forceps. The plates were then incubated at 37°C for 24 hours. Following the 2017 AST guidelines established by the Clinical Laboratory Standards Institute (CLSI), the inhibition zones around each antibiotic disc were measured in millimeters using a ruler.

CHAPTER FOUR

RESULT

The results for the total heterotrophic bacterial counts obtained from the fecal samples of poultry birds are presented in **Table 4.1** below. The bacterial counts ranged from 1.00×10^6 CFU/g to 65.00×10^6 CFU/g across the different samples and dilutions. The highest total heterotrophic bacterial count was observed in **sample F15**, which recorded a values of 65.00×10^6 CFU/g, while the lowest count was recorded in **sample F7**, with values of 1.0×10^6 CFU/g. **sample F9** recorded no growth. The morphological, cultural, and biochemical characteristics of bacterial isolates from the fecal samples are shown in **Table 4.2.** A total of five distinct bacterial species were identified based on colony appearance, staining reactions, and biochemical profiles. All the

isolates were Gramnegative rods. The bacterial species identified were *Escherichia coli*, *Salmonella sp.*, *Klebsiella pneumonia.*, *Proteus mirabilis.*, *Klebsiella oxytoca.*

The frequency and percentage occurrence of the bacterial isolates from the fecal samples of poultry birds are shown in **Table 4.3.** Among the identified bacterial species, *Escherichia coli* had the highest frequency of occurrence (53.33%), followed by *Klebsiella pneumoniae* and *Salmonella sp.* with frequencies of (46.67%) and (26.67%) respectively. *Klebsiella oxytoca* had a frequency of (20.00%) while *Proteus mirabilis* had the lowest frequency of (6.67%).

Table 4.1: The average bacterial count (CFU/g) in the poultry droppings (fecal samples)

Sample	**Total Bacterial Counts ($\times 10^6$ CFU/g)**
F1	9.50
F2	20.00
F3	62.50
F4	9.00
F5	40.00
F6	2.50
F7	1.00

F8	5.00
F9	-
F10	4.00
F11	15.00
F12	10.50
F13	9.00
F14	17.50
F15	65.00
Mean value	18.03

Key: F= feaces

CFU/g= colony forming unit/gram

Table 4.2: Cultural, Morphological, and Biochemical Characteristics of Bacterial Isolates from Fecal Samples of Poultry

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Elevation	Flat	Raised	Raised	Flat	Flat
Margin	Entire	Entire	Entire	Entire	Entire
Texture	Wet	Dry	Wet	Wet	Wet
MacConkey (Agar 1)	pink	Pale	Pink	Pale	Pink
SSA (Agar 2)	Pink colonies with black black center	Pale with center	Pink	Colourless	Pink
EMB (Agar 3)	Metallic green sheen	Colourless	Mucoid pink	Colourless	Mucoid purple

Shape	Circular	Circular	Circular	Circular	Circular
Size	Medium	Large	Large	Medium	Medium
Gram Stain	–	–	–	–	–
Cell Type	Rod	Rod	Rod	Rod	Rod
Arrangement	Single	Pair	Single	Single	Single
Color (Gram Reaction)	Pink	Pink	Pink	Pink	Pink
Catalase	+	+	+	+	+
Indole	+	–	–	–	+
Citrate	–	+	+	+	+
Oxidase	–	–	–	–	–
Urease	–	+	+	+	+
Identity	*Escherichia coli*	*Salmonella sp.*	*Klebsiella pneumonia*	*Proteus mirabilis*	*Klebsiella oxytoca*

Key: + = Positive to test

– = Negative to test

Table 4.3: Frequency and Percentage Occurrence of Bacterial Isolates from the Total Fecal Samples of poultry (15 samples)

Bacterial Isolates	**Number of Occurrences (n=15)**	**Frequency (%)**
Escherichia coli	8	53.33
Klebsiella pneumoniae	7	46.67
Salmonella sp.	4	26.67
Klebsiella oxytoca	3	20.00

Proteus mirabilis	1	6.67

Table 4.4. presents the frequency and isolation rate of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Salmonella* spp., and *Proteus mirabilis* from pooled fecal samples collected from different poultry types, genders, and age groups within Benin City. The combine result of *Klebsiella pneumoniae* and *Klebsiella oxytoca* showed the highest occurrence among the isolates, particularly in broilers, while *E. coli* was recovered across all bird types. Variations were also observed between male and female birds, as well as between younger (≤3 months) and older (≥4 months) birds, indicating that factors such as bird type, age, and sex may influence the distribution of enteric bacteria in poultry populations.

The results of the antibiotic susceptibility test for bacterial isolates from the fecal samples of poultry birds are presented in **Table 4.5.** The antibiotic sensitivity patterns varied among the bacterial isolates. *Escherichia coli* exhibited moderate resistance to Gentamycin (62.50%) and Tarivid (50.00%). A low sensitivity to Ciprofloxacin (12.50%) and a (100.00%) resistance to Sparfloxacin, Amoxicillin, Augmentin and Pefloxacin. *Salmonella sp.* isolates showed moderate sensitivity to Tarivid (50.00%) and showed low sensitivity to Ciprofloxacin (25.00%) but were completely resistant to Sparfloxacin, Amoxicillin, Augmentin, Gentamycin and Pefloxacin (100.00%). *Klebsiella oxytoca* exhibited (100.00%) sensitivity to Gentamycin and Tarivid. A low sensitivity to Ciprofloxacin (33.33%) but complete resistance to Sparfloxacin, Amoxicillin, Augmentin and Pefloxacin (100.00%). *Klebsiella pneumoniae* showed low resistance to Ciprofloxacin (14.29%), Tarivid (42.86%), Gentamycin (28.57%). A complete resistance to Sparfloxacin, Amoxicillin, Augmentin and pefloxacin (100.00%). *Proteus sp.* displayed complete resistance (100.00%) to all the antibiotics used.

Table 4.4: Frequency (%) and Isolation Rate of *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Proteus mirabilis* from pooled fecal samples from various location in Benin city.

Variable	**Category**	No. of examined sample (n=15)	*Escherichia coli*	*K. pneumoniae and K. oxytoca*	*Salmonella sp.*	*Proteus mirabilis*
Types of birds	Broilers	12	5(41.66)	10(83.33)	3(25.00)	1(8.33)
	Layers	2	2(100.00)	0(0.00)	0(0.00)	0(0.00)
	Cockerels	1	1(100.00)	0(0.00)	1(50.00)	0(0.00)
Gender	Male	8	3(37.5)	7(87.50)	2(25.00)	0(0.00)
	Female	7	5(71.43)	3(42.86)	2(28.57)	1(100.00)
Age	≤ 3 months	13	6(46.15)	10(76.92)	4(30.77)	1(7.69)
	≥ 4 months	2	2(100.00)	0(0.00)	0(0.00)	0(0.00)

Table 4.5: Antibiotic Sensitivity Test of Bacterial Isolates from Fecal Samples of Poultry

Bacterial Isolates	SP	CPX	AM	AU	CN	PEF	OFX
Escherichia coli (n=8)	0(0.00)	1(12.50)	0(0.00)	0(0.00)	5(62.50)	0(0.00)	4(50.00)
Salmonella sp. (n=4)	0(0.00)	1(25.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(50.00)
Klebsiella pneumonia (n=7)	0(0.00)	1(14.29)	0(0.00)	0(0.00)	2(28.57)	0(0.00)	3(42.86)
Klebsiella oxytoca (n=3)	0(0.00)	1(33.33)	0(0.00)	0(0.00)	3(100.00)	0(0.00)	3(100.00)
Proteus mirabilis. (n=1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

KEY:

SP: Sparfloxacin (10 µg); CPX: Ciprofloxacin (30 µg); AM: Amoxicillin (10 µg); AU: Augmentin (10 µg); CN: Gentamycin (30 µg); PEF: Pefloxacin (30 µg); OFX: Tarivid (10 µg).

Figure 4.1. shows the percentage occurrence of multi-drug resistant (MDR) strains. *Proteus mirabilis* showed (100.00%) multi-drug resistance, followed by *Salmonella sp*. (50.00%), *Klebsiella pneumoniae* (42.80%), and *Escherichia coli* (37.50%).

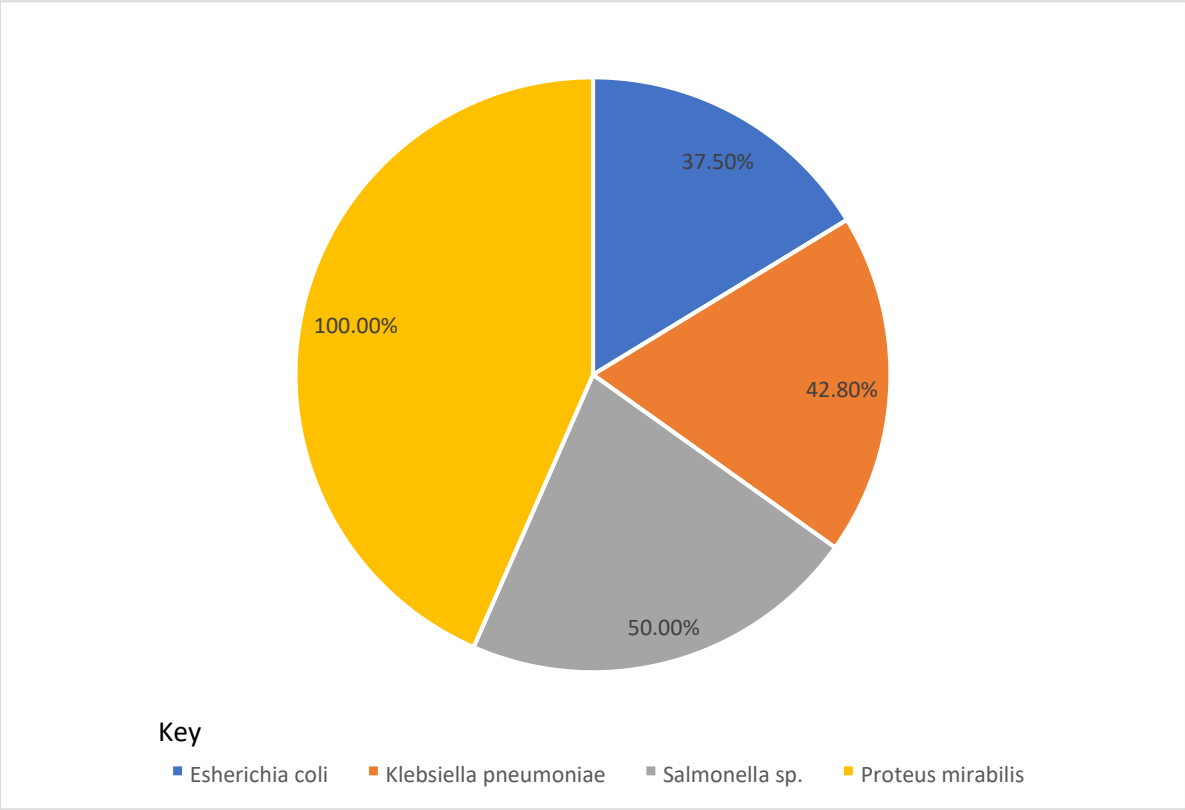

Figure 4.1: Percentage Occurrence of Multi-drug Bacterial Isolates

CHAPTER FIVE

DISCUSSION

Poultry encompasses domesticated bird species reared primarily for the production of eggs, meat, and feathers (Getu & Tadese, 2015; Wilson, 2021). In Nigeria, poultry farming has become one of the most important livestock enterprises, experiencing substantial expansion in both commercial and backyard production systems. In metropolitan areas such as Benin City, poultry production plays a critical role in meeting the increasing demand for animal protein through the supply of eggs and poultry meat. Despite these economic and nutritional benefits, poultry birds are recognized reservoirs of numerous zoonotic and foodborne bacterial pathogens capable of causing disease in humans and animals (Ifeanyichukwu et al., 2016).

The present study demonstrated considerable bacterial contamination in poultry fecal samples, with total heterotrophic bacterial counts ranging from 1.00×10^6 CFU/g to 65.00×10^6 CFU/g. These elevated microbial loads indicate substantial bacterial colonization within the gastrointestinal tract of the birds and may reflect differences in management practices among poultry farms. Variations in bacterial counts could be attributed to factors such as feed quality, sanitation status, stocking density, waste management practices, and environmental hygiene. Previous studies have identified poultry feed as a potential source of microbial contamination capable of introducing bacteria into poultry production systems (Uwaezuoke & Ogbulie, 2008). Consequently, contaminated feed may contribute to increased bacterial shedding in poultry droppings and may also pose health risks through the transmission of pathogenic microorganisms and their toxins, as highlighted by Ramos et al. (2020).

Cultural, morphological, and biochemical characterization of isolates recovered from poultry fecal samples led to the identification of five bacterial species, namely Escherichia coli, Salmonella spp., Klebsiella pneumoniae, Klebsiella oxytoca, and Proteus mirabilis. All isolates were Gram-negative rods, a

characteristic feature of members of the family Enterobacteriaceae and other enteric bacterial groups. The bacterial profile observed in this study is comparable to the findings of Umar et al. (2023), who reported the occurrence of Escherichia coli, Shigella spp., Proteus mirabilis, and Salmonella spp. in poultry-associated samples. The current investigation further confirms the widespread occurrence of Salmonella spp., Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, and Proteus mirabilis within poultry environments.

Among the identified isolates, Klebsiella species exhibited the highest occurrence rate (66.67%), followed by Escherichia coli (53.33%), Salmonella spp. (26.67%), and Proteus mirabilis (6.67%). The predominance of Klebsiella species agrees with the findings of Jesumirhewe et al. (2023), who similarly reported frequent isolation of Klebsiella spp. and Escherichia coli from poultry farms in Nigeria. The high prevalence of these organisms may be linked to fecal contamination, poor sanitation practices, and the ability of these bacteria to persist within poultry production environments.

Similarly, Bamidele et al. (2022) reported a high prevalence of Escherichia coli and Salmonella spp. in poultry droppings collected from the Guinea Savannah region of Nigeria. The occurrence of these organisms reinforces their status as common enteric bacteria associated with poultry and highlights their potential role in zoonotic transmission. The possibility of genetic similarities between poultry and human strains further raises public health concerns regarding the transmission of antimicrobial-resistant pathogens through the food chain. Although Escherichia coli is generally regarded as a normal component of the intestinal microbiota, pathogenic strains have been implicated in both avian and human diseases. In poultry, pathogenic E. coli strains are associated with colibacillosis, which may manifest as localized enteric infections or systemic septicaemic disease (Park et al., 2010). Likewise, enterohaemorrhagic strains such as verocytotoxin-producing E. coli O157:H7 are important zoonotic pathogens capable of causing severe gastroenteritis and haemorrhagic colitis in humans.

Analysis of bacterial distribution according to bird type revealed notable variations in isolation frequencies. Klebsiella pneumoniae and Klebsiella oxytoca recorded the highest occurrence among broilers (83.33%), followed by Escherichia coli (41.66%) and Salmonella spp. (25.00%), whereas Proteus mirabilis exhibited the lowest occurrence (8.33%). In contrast, Escherichia coli was isolated from all sampled layers (100.00%) and the single cockerel examined (100.00%). The higher occurrence of enteric bacteria among layers may be associated with prolonged exposure to contaminated litter, feed, and water sources resulting from their longer production lifespan compared with broilers.

Gender-based analysis revealed a higher prevalence of Klebsiella pneumoniae and Klebsiella oxytoca among male birds (87.50%), whereas Escherichia coli (71.43%) and Proteus mirabilis (100.00%) were more frequently isolated from female birds. The increased prevalence of E. coli among female birds may be associated with physiological and hormonal factors influencing intestinal microbial ecology, as well as increased fecal shedding during egg production cycles, as suggested by Olawale et al. (2019).

Age-related distribution showed that birds aged three months or younger harbored a greater diversity of enteric bacteria, including Escherichia coli (46.15%), Klebsiella pneumoniae and Klebsiella oxytoca (76.92%), Salmonella spp. (30.77%), and Proteus mirabilis (7.69%). In contrast, birds aged four months and above yielded only Escherichia coli (100.00%). The predominance of E. coli in older birds may indicate the establishment of a relatively stable intestinal microbiota dominated by commensal and opportunistic strains. This observation supports the findings of Ehinmidu et al. (2021), who reported increased persistence of E. coli with advancing age due to prolonged exposure to contaminated environmental sources, including drinking water and poultry housing facilities.

The antimicrobial susceptibility profiles obtained in this study revealed varying levels of resistance among the bacterial isolates. Escherichia coli exhibited low susceptibility to ciprofloxacin (12.50%) and moderate susceptibility to gentamicin (62.50%) and ofloxacin (50.00%), while complete resistance (100.00%) was observed against sparfloxacin, amoxicillin, augmentin, and pefloxacin. Similarly,

Salmonella spp. demonstrated limited susceptibility to ciprofloxacin (25.00%) and ofloxacin (50.00%), but complete resistance to sparfloxacin, amoxicillin, augmentin, gentamicin, and pefloxacin. Klebsiella pneumoniae displayed low susceptibility to ciprofloxacin (14.30%) and pefloxacin (28.60%), moderate susceptibility to ofloxacin (42.90%), and complete resistance to β-lactam antibiotics and gentamicin. Klebsiella oxytoca exhibited complete resistance to sparfloxacin, amoxicillin, augmentin, and gentamicin, while maintaining partial susceptibility to ciprofloxacin (33.30%) and complete susceptibility to pefloxacin and ofloxacin (100.00%). Notably, Proteus mirabilis demonstrated resistance to all antimicrobial agents tested, indicating a pan-resistant phenotype. Overall, more than 80% of the isolates displayed resistance to multiple antibiotic classes, underscoring the widespread occurrence of antimicrobial resistance among enteric bacteria recovered from poultry fecal samples.

Further analysis identified multidrug-resistant (MDR) isolates based on resistance to at least three antimicrobial classes, including β-lactams, aminoglycosides, and fluoroquinolones. The proportions of MDR isolates recorded were 37.50% for Escherichia coli, 42.86% for Klebsiella pneumoniae, 50.00% for Salmonella spp., and 100.00% for Proteus mirabilis. These findings corroborate reports by Ejeh et al. (2017), who documented multidrug resistance among Escherichia coli and Salmonella isolates recovered from broilers and indigenous chickens in Zaria, Nigeria, particularly against β-lactam and fluoroquinolone antibiotics. Similarly, Cookey and Otokunefor (2016) reported high resistance rates among poultry-associated bacterial isolates from southern Nigeria, with multidrug resistance exceeding 80% across aminoglycoside and β-lactam classes. Comparable observations were also made by Bamidele et al. (2022), who reported substantial resistance to quinolones, aminoglycosides, and β-lactams among enteric bacteria isolated from poultry droppings in the Guinea Savannah zone of Nigeria.

The high prevalence of multidrug-resistant bacteria observed in this study may be attributed to the indiscriminate use of antibiotics in poultry production for prophylactic, therapeutic, and growth-promoting purposes. Such practices create selective pressure that favours the survival and proliferation of

resistant bacterial populations. Continuous exposure to sub-therapeutic concentrations of antimicrobial agents enhances the acquisition and dissemination of resistance determinants through horizontal gene transfer mechanisms involving plasmids, transposons, and other mobile genetic elements (Oloso et al., 2018; Aworh et al., 2019). These genetic mechanisms likely contributed to the multidrug-resistant phenotypes observed among the isolates recovered in the present investigation.

Furthermore, Salihu et al. (2014) highlighted the widespread availability, affordability, and uncontrolled use of antibiotics within poultry production systems as major drivers of antimicrobial resistance. Ejeh et al. (2017) similarly identified excessive antibiotic usage, poor farm sanitation, overcrowding, and inadequate biosecurity practices as key factors promoting the emergence and dissemination of resistant bacterial strains. Collectively, these factors may explain the increasing prevalence of antimicrobial-resistant enteric bacteria in Nigerian poultry farms and underscore the urgent need for antimicrobial stewardship, improved biosecurity measures, and continuous surveillance to mitigate the spread of resistance within poultry production systems and the wider environment.

5.2. Conclusion

The findings of this study demonstrated that poultry birds in Benin City harbor multiple zoonotic bacterial species, some of which exhibit multidrug resistance. These results corroborate earlier reports across Nigeria and other African countries that have identified poultry birds as important reservoirs of enteric and antibiotic-resistant pathogens. The relatively high bacterial load and resistance rates observed suggest that poultry birds and its products could play a critical role in the dissemination of resistant bacteria within communities which carries a significant implications for both the economy and public health.

5.3 Recommendations

1. Poultry stocking density should be maintained at optimal, moderate levels to prevent overcrowding, as excessive stocking increases stress, enhances fecal contamination, and facilitates rapid transmission of infectious agents among birds.

2. Feeders and drinkers should be cleaned and disinfected on a daily basis, with consistent provision of clean, uncontaminated feed and potable water to minimize the risk of microbial proliferation and ingestion of pathogens.

3. Strict hygiene and sanitation protocols should be consistently implemented within poultry production units to reduce environmental microbial load, particularly in litter, feed, and droppings, thereby limiting opportunities for pathogen survival and spread.

4. Poultry farmers should be adequately trained and sensitized on proper waste management practices, including safe disposal of droppings and litter, to prevent environmental contamination, cross-contamination within housing systems, and deterioration of feed quality.

5. The use of antimicrobial agents in poultry production should be carefully regulated and monitored, with emphasis on prudent application guided by veterinary oversight, in order to reduce selective pressure and curb the emergence and spread of antimicrobial-resistant bacteria.

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