Prevalence, characterization and antimicrobial susceptibility patterns of extraintestinal pathogenic *Escherichia coli* from chicken carcasses in Gauteng Province, South Africa

by

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DECLARATION

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I, Mmatau Carol Motau, thus certify that the dissertation I submitted to the University of South Africa for a Master of Science in Life Science degree is entirely original with no submissions to this or any other higher education institution. I additionally declare that a thorough list of references is used to indicate and credit all sources mentioned or quoted.

Signature:  Date: 01/12/2025

DEDICATION

I dedicate this dissertation to my loving husband and son whose patience, understanding, sacrifice and love have been invaluable throughout this challenging journey. To my parents and siblings whose unwavering support and encouragement have been my guiding light throughout this journey making this achievement possible. Your influence has shaped me into the person I am today. To God Almighty whose divine guidance, wisdom and strength have sustained me throughout this journey. Your grace has been my solace and I am eternally grateful.

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains are adaptable bacteria often associated with urinary tract, kidney, bloodstream and other extraintestinal infections. Extraintestinal pathogenic *Escherichia coli* infections pose a global public health threat due to their economic impact and management challenges, with poultry meat and other meat products implicated in the spread of the disease. However, in South Africa, there is scarcity of published data on the contamination level and role that poultry meat plays in transmitting ExPEC. The purpose of the study was to determine the prevalence, genetic characteristics and antimicrobial susceptibility profile of ExPEC from chicken carcasses sampled from the Gauteng Province. The study utilized a stratified proportional sampling method to analyse 404 chicken carcasses collected from four abattoirs across five municipalities in Gauteng Province. An additional 63 historically persevered freeze-dried isolates from poultry were included to strengthen this study. Most Probable Number method was used for detection of *Escherichia coli*. Antimicrobial susceptibility testing was conducted using the minimum inhibition concentration method against 12 antibiotics. Selected classes of antibiotics include cephalosporin, macrolide, phenicol, tetracycline, penicillin, fluoroquinolone, aminoglycoside, and sulphonamide. This selection is motivated by the necessity to comprehend, monitor, and address the emergence of resistance in a common Gram-negative bacteria including ExPEC. Conventional polymerase chain reaction was used to confirm ExPEC presence, alongside gene characterization through virulence genotyping, phylogenetic grouping, phenotypic resistance, and pathotyping. The overall prevalence of *E. coli* was 93% with 4% confirmed as ExPEC. The ExPEC isolates predominantly carried multiple virulence genes: *ompA* (100%), *mat* (83%), *kpsm* (65%), both *csg* and *sitA* with (57%), *sat* and *traT* (52%) virulent genes. The highest antibiotic-resistant rates were observed in tetracycline-32-4mg/L (57%), ciprofloxacin- 32-4mg/L (43%), and gentamicin-16-0.25mg/L (39%). However, ceftriaxone, ceftiofur, and cefoxitin (9%) exhibited the lowest antibiotic resistance. The genes associated with the highest phenotypic resistance were *sul3* (43%) and *tetA* (39%). The phylogenetic groups indicated that majority of the ExPEC isolates belonged to group A (39%), group B2 (35%) lastly both groups B1 and D (13%). Class 3 integron was detected in 74% isolates followed by Class 1 integron (35%) and Class 2 (9%). The NMEC pathotype was rare among these isolates, while 13% and 26% distribution were observed in APEC, UPEC, and SEPEC pathotypes. In conclusion, it is evident that while ExPEC occurrence is rare, *E. coli* is consistently isolated from chicken meat. Despite its low frequency, ExPEC demonstrated a high prevalence of Class 3 integron and virulence-encoding

genes that are crucial for colonization and survival. These factors contribute to the spread of resistance genes and emergence of multidrug resistance. Future research should utilize whole genome sequencing for comprehensive molecular characterization, focusing on plasmids and serotyping to differentiate strains from formal and informal abattoirs, which may reveal sources of contamination.

Keywords: Virulence genes; Resistance genes; Minimum Inhibition Concentration; abattoir

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LIST OF ABBREVIATIONS

AMR Antimicrobial Resistance
ARC Agriculture Research Council
ATCC American Type Culture Collection
APEC Avian Pathogenic *E. coli*
API Analytical Profile Index
BBB Blood-Brain Barrier (BBB)
bp Base pairs
BG Brilliance Green Broth
BHI Brain Heart Infusion
CC Clonal Complexes
CNS Central nervous system
DAEC Diffusely adherent *E. coli*
DNA Deoxyribonucleic acid
EAEC Enteroaggregative *E. coli*
EIEC Enteroinvasive *E. coli*
EHEC Enterohemorrhagic *E. coli*
EMB Eosin Methyl Brilliance Agar
EnPEC Endometrial Pathogenic *E. coli*
E. coli *Escherichia coli*
ETEC Enterotoxigenic *E. coli*
ExPEC Extraintestinal pathogenic *E. coli*
EPEC Enteropathogenic *E. coli*
EUCAST European Committee on Antimicrobial Susceptibility Testing
FDA Food and Drug Administration
FW Forward
G Gas
g Gram
hrs hours
IPEC Intestinal Pathogenic *E. coli*
ISO International Standard Organization
MDR Multidrug Resistance
Mg MicroGram

µl	Microlitre
µm	Micrometre
µM	Micromolar
mg	MilliGram
min	Minutes
MIC	Minimum Inhibitory Concentration
mM	Millimolar
ml	Millilitre
MLST	Multi-locus Sequence Typing
MPEC	Mammary Pathogenic *E. coli*
mPCR	multiplex Polymerase Chain Reaction
MPN	Most Probable Number
NMEC	Neonatal Meningitis *E. coli*
O	Somatic antigen
OmpA	Outer membrane protein A
OVI	Onderstepoort Veterinary Institute
PDR	Pan Drug Resistant
PCR	Polymerase Chain Reaction
qPCR	Real time PCR
QS	Quorum sensing
RNA	Ribonucleic acid
SA	South Africa
sec	Seconds
SHS	Swollen Head Syndrome
SEPEC	Septicemic *E. coli*
ST	Sequence Type
T	Turbidity
UPEC	Uropathogenic *E. coli*
US	United States
USA	United States of America
UTI	Urinary tract infection
UV	Ultraviolet
V	Volts
VF	Virulent factors

VG Virulent groups

WGS Whole Genome Sequencing

WHO World Health Organisation

LIST OF SYMBOLS

℃ Degree Celsius

µl microliter

% Percentage

CHAPTER 1: INTRODUCTION

1.1 Background

Escherichia coli (*E. coli*) is a ubiquitous bacterium commonly found in the gut of warm-blooded mammals including animals and humans (Jang et al., 2017). Most *E. coli* strains are harmless commensals that rarely cause disease and coexist in beneficial interactions with their hosts (Ramos et al., 2020). The role of *E. coli* is primarily beneficial in aiding digestion and vitamin synthesis (such as Vitamins K and B), which supports intestinal health and inhibits harmful bacteria. It also influences host immune development and regulation (Zhan, 2025). While most *E. coli* strains are harmless, some can cause serious illnesses in both animals and humans (Sora et al., 2021). In humans, pathogenic *E. coli* strains primarily affect the elderly, young children, and immunocompromised individuals, leading to conditions such as colibacillosis, sepsis, inflammatory bowel disease, neonatal meningitis, and urinary tract infections (UTIs) (Percival & Williams, 2014; Villavicencio-Carrisoza et al., 2025). Similarly, in animals particularly chickens, *E. coli* induces colibacillosis, which manifests coligranuloma, synovitis, peritonitis, salpingitis, and air sacculitis (Jakaria et al., 2013).

The pathogenicity of *E. coli* is broadly categorized into two major groups based on the site of infection: intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Sora et al., 2021; Pokharel et al., 2023). The IPEC strains are primarily responsible for gastrointestinal infections and are typically transmitted via the fecal-oral route, often through contaminated food or water (Govindarajan et al., 2020; Paul, 2024). These strains are further divided into various pathotypes based on their virulence factors, clinical symptoms, and mechanisms of pathogenesis (Aijuka & Buys, 2019). In contrast, ExPEC strains can cause infections outside the gastrointestinal tract and are often associated with serious systemic diseases (Sora et al., 2021). These include UTIs, neonatal meningitis, sepsis, and respiratory infections (Smith et al, 2007; Meena et al., 2023; Pokharel et al., 2023). The ExPEC is further classified into five main pathotypes namely avian pathogenic *E. coli* (APEC), neonatal meningitis *E. coli* (NMEC), septicaemic *E. coli* (SEPEC) and uropathogenic *E. coli* (UPEC). Many ExPEC strain have been frequently isolated from poultry, particularly chickens and chicken meat, raising concerns about zoonotic transmission and the role of poultry as a reservoir for human infections (Mitchell et al., 2015). Understanding the pathotypic diversity

of *E. coli* is critical for epidemiological surveillance, risk assessment, and the development of targeted interventions to reduce transmission and disease burden in both human and animal population.

The global consumption and production of chicken meat have grown significantly over the past decade and is predicted to continue increasing. For instance, South Africans consume over 60% (23 million chickens a week), totalling nearly 1.2 billion chickens per year of total meat consumption in the country with local production of 1.9 million tonnes (SAPA, 2022). This surge is largely due to chicken's relative affordability compared to other meats (Birhan, 2014; Birhanu et al., 2023). However, the potential for chicken meat to carry pathogenic strains of *E. coli* throughout the meat value chain is well documented (Wardhana et al., 2021; Klaharn et al., 2022; Ranasinghe et al., 2022; Mathew et al., 2025). Given its susceptibility to bacterial contamination, including ExPEC, chicken meat poses a concern for public health (Zou et al., 2021; Meena et al. 2023).

Over the past decade, ExPEC has garnered significant research and surveillance attention interest from international food industries and governmental organizations (Mellata, 2013; Manges, 2016; Meena et al., 2023; Guragain et al., 2025). This heightened focus stems from its public health significance, given its high morbidity and mortality (10% in chickens, 9%-12% in human) rates, as well as its socio-economic implications (Geurtsen et al., 2022). Genetic similarities have been found between human ExPECs, such as UPEC and NMEC, and avian ExPECs (APEC), specifically in virulent plasmid genes (*hlyF, iroN, iss*), this indicates that poultry may represent a zoonotic source of ExPEC infections in humans due to their close genetic relationship (Mellata, 2013; Manges, 2016; Sarowska et al., 2019; Kathayat et al., 2021; Watts & Wigley, 2024). This pose a considerable risk for countries like South Africa (SA), where a significant portion of the population on chicken meat as a primary source of high-quality animal protein (Nkukwana, 2019).

The escalation of antibiotic resistance has significantly complicated the treatment of infections caused by ExPEC, particularly since the post 1990s (Longhi et al., 2022). Extraintestinal pathogenic *E. coli* infections are commonly treated with cephalosporins, fluoroquinolones and trimethoprim-sulfamethoxazole (Pitout, 2012). However, resistance to these antibiotics leads to delays in effective therapy, increasing morbidity and mortality (Pitout, 2012; Dadgostar, 2019). Up until the late 1990s, ExPEC strains were generally susceptible to first line antibiotics,

but surveillance studies conducted in Europe, North America, and South America during 2000s revealed that between 20 and 45% of ExPEC strains had developed resistance to cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (Pitout, 2012; Biswas et al., 2024). The main cause of resistance to the antibiotics is the production of extended-spectrum beta-lactamases, this is often driven by misuse of antibiotics in healthcare system to treat *E. coli* and as growth promoters in the agriculture sector giving ExPEC an evolutionary resistant advantage (Sora et al., 2021). These findings align with a recent study conducted in SA in 2020 which examined antibiotic resistance from humans and pigs (Founou et al., 2020). Therefore, it is essential to investigate the prevalence, characterize the diversity, and antibiotic resistance patterns of ExPEC in chickens within the country, highlighting the need for the proposed study.

1.2 Problem statement

Extraintestinal pathogenic *E. coli* strains have been isolated from a variety of sources, including animal, human, food, environment, and water (van El et al., 2013; Sarowska et al., 2019; Sora et al., 2021). Research indicates similarities in clonal lineages and virulence genes between human and animal derived ExPEC isolates, both of which can adhere to and invade human intestinal epithelial cells (Clermont et al., 2011; Massella et al., 2021; Zou et al., 2021). This suggest that food of animal origin may be a potential route of transmission for ExPEC infections in humans. In addition, ExPEC strains have also been detected in the feces of healthy chickens, and it is hypothesised that contamination of chicken carcasses during slaughter may occur due to gastrointestinal rupture or other processing-related factors (Sarowska et al., 2019; Marmion et al., 2021). Importantly, these ExPEC isolates can cause human infections, contributing to morbidity and mortality, increased healthcare costs, significant economic costs, including lost productivity (Poolman & Wacker, 2016; Manges et al., 2019).

Another public health threat is the existence of antibiotic resistance among foodborne microorganisms as identified by the WHO in 2019 (WHO 2021). Furthermore, ExPEC has raised concerns about the potential acquisition of antimicrobial resistant genes in livestock environments, potentially making animal and human infections more difficult and costly to treat (Harbarth et al., 2015). Several studies have demonstrated that genes producing extended-spectrum β-lactamases found in isolates of ExPEC from chicken are responsible for antibiotic

resistance reducing treatment options and increase morbidity due to increased ExPEC infections (Da Costa et al., 2013; Longhi et al., 2022).

Extraintestinal pathogenic *E. coli* strains harbour various virulence factors (adhesin: *pap/fimH*, toxins: *hlyA/cnf1* iron acquisition: *iucD*) responsible for pathogenesis, often encoded by virulence islands and DNA elements, enabling disease manifestation outside the intestinal tract (Sarowska et al., 2019). These virulence factors aid ExPEC to facilitate their invasion and evasion of the host's defence mechanisms, exacerbating pathogenesis (Sora et al., 2021). The accumulation of virulent genes heightens pathogenicity and disease severity. Gauteng is one of the most densely populated provinces in South Africa (SA) with a high consumption of poultry products. As such, it is important to study zoonotic transmissions such as ExPEC. Research in Gauteng has brought attention to the high prevalence of pathogens such as *Salmonella, Campylobacter* and *Listeria monocytogenes* in small-scale broiler abattoirs, as well as concerns about food safety, hygiene practices, and possible pathogen contamination, this makes it a relevant location for investigating ExPEC in chicken abattoirs (Adesiyun et al., 2020; Amar, 2022). Given the public health and economic implications of ExPEC, it is concerning that limited research has been conducted on its prevalence and genetic diversity in chickens within SA. This highlights the urgent need for enhanced surveillance and monitoring of ExPEC in animals and animal-derived food products.

1.3 Aims and objectives

1.3.1 Aim

The aim of this study aim was to provide epidemiological data on the distribution and characterization of Extraintestinal pathogenic *E. coli* in broiler chicken carcasses from Gauteng Province.

1.3.2 Objectives

The objectives of the study were to:

1. Investigate the prevalence of ExPEC in broiler chicken carcasses and their associated abattoirs in Gauteng, SA.
2. Ascertain the phenotypic and genotypic antibiotic resistance profiles of ExPEC isolated from broiler chicken carcasses in Gauteng, SA.

3. Identify the virulence genes associated with ExPEC from broiler chicken carcasses in Gauteng, SA.

1.4 Research questions

a) What is the prevalence of ExPEC in broiler chicken carcasses in Gauteng, SA?
b) Which pathotypes of ExPEC are associated with broiler chicken carcasses in Gauteng, SA?
c) What are the phenotypic patterns observed in ExPEC isolates from broiler chicken carcasses in Gauteng, SA?
d) What is the genotypic trait of ExPECstrains associated with broiler chicken carcasses in Gauteng, SA?
e) Which virulent genes are present in ExPEC isolates from broiler chicken carcasses in Gauteng, SA?

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to literature review

Extraintestinal pathogenic *E. coli* is a notable pathogen that causes infections beyond the gastrointestinal tract, facilitated by virulent factors that promote tissue colonization. Transmission occurs through contaminated food, water, and animals, leading to diverse clinical manifestations in both humans and animals (Sora et al., 2021). Their pathogenesis involves complex interactions between bacterial virulence factors and host immune responses, with strains classified into four main phylogenetic groups (A, B1, B2, and D), where B2 and D, are most associated with extraintestinal infections (Čurová et al., 2020). Key ExPEC virulence traits include adhesins, toxins, and iron acquisition systems (Qiu et al., 2024). Antibiotic resistance complicates treatment, necessitating effective prevention strategies. Integrons contribute to the spread of antibiotic resistance among ExPEC strains (Sabbagh et al., 2021). Methods for isolating and identifying ExPEC, including traditional culture and molecular techniques, are crucial for understanding its epidemiology and pathogenesis, although their accuracy and specificity vary. This review addresses the characteristics, transmission, and pathogenesis of ExPEC, emphasizing the importance of this knowledge for developing effective control measures. It discusses current methods for ExPEC isolation and identification, along with their strengths, limitations, and applications.

2.2 General characteristics of *Escherichia coli*

Escherichia coli is widely distributed in nature and found in diverse niches including food, water, the environment and the intestines of both humans and animals (poultry, bovine, pigs, ovine etc) (Rasheed et al., 2014). The genus *Escherichia* is named after Theodor Escherich, as honour for discovering this pathogen (Gomes et al., 2016). Taxonomically, *E. coli* belongs to the genus *Escherichia*, family *Enterobacteriaceae*, order Enterobacterials, class Gamma proteobacteria, and phylum Proteobacteria (Gomes et al., 2016). Currently, there are six recognized species within the genus *Escherichia* (McVey, Kennedy, & Czuprynski, 2022). These species include *E. albertii, E. fergusonii, E. hermanii, E. vulneris. E. adecarboxylata* and *E. blattae*. However, *E. adecarboxylata* and *E. blattae* have been reclassified as *Leclercia adecarboxylata* and *Shimwellia blattae* respectively (Ramos et al., 2020; McVey, Kennedy, &

Czuprynski, 2022). Despite the fluctuating position of the other members of the genus, phylogenetic and biochemical data continue to point to *E. coli* as the most common species that is widely recognized as a cause of serious diseases in both humans and animals (Ramos et al., 2020).

Escherichia coli is a Gram-negative, nonsporulating coliform bacterium that is often found in the lower intestine of warm-blooded species (Basavaraju & Gunashree, 2023; Malabadi et al., 2024). Cells are generally rod-shaped, measuring around 2.0µm in length, 0.25µm to 1µm in diameter, and 0.6µm³ to 0.7µm³ in volume. This bacterium is peritrichous in structure and possess flagella for motility (Percival & Williams, 2014; Nedeljković et al., 2021).

Escherichia coli grows best at an optimal temperature of 37°C with minimum of 10°C and maximum of 42°C, while some strains may grow as high as 49°C (Basavaraju & Gunashree, 2023). It is a facultative anaerobe when oxygen is accessible and present, it uses oxidative metabolism. However, it still thrives in the absence of oxygen by utilizing anaerobic respiration or fermentation (von Wulffen et al., 2016). *Escherichia coli* can adapt to various environmental conditions with pH levels ranging from 4.5 to 9.5, optimal at 7.0 pH, and tolerates salt concentrations of 0.5-1% sodium chloride, with a minimum of 0% and a maximum of 10%. It thrives at optimal moisture content of 0.95 aW (Medvedova et al., 2021; Dhakar & Pandey 2016).

2.3 Pathotypes of *Escherichia coli*

Escherichia coli is primarily classified into three different types depending on its pathogenic potential, namely, commensal *E. coli* (non-pathogenic), intestinal pathogenic *E. coli* (IPEC), and extraintestinal pathogenic *E. coli* (ExPEC) (Ramos et al., 2020). The IPEC is further divided into six pathotypes (Figure 1). These are; (1) enteropathogenic *E. coli* (EPEC), which causes diarrhoea in humans and animals; (2) enterohemorrhagic *E. coli* (EHEC), which causes hemorrhagic colitis and hemolyticuremic syndrome; (3) enterotoxigenic *E. coli* (ETEC), which is the main cause of traveller's diarrhoea, porcine diarrhoea, and bovine diarrhoea; (4) diffusely adherent *E. coli* (DAEC), a subtype of enteroaggregative *E. coli* that causes diarrhoea in children; (5) Enteroaggregative *E. coli* (EAEC), which can cause chronic diarrhoea in human; (6) Enteroinvasive *E. coli* (EIEC), which can cause watery diarrhoea and gastroenteritis (Stromberg et al., 2017; Pokharel, Dhakal & Dozois 2023).

Extraintestinal pathogenic *E. coli* is also divided into four pathotypes (Figure 1): namely, (1) uropathogenic *E. coli* (UPEC), causing infection in the urinary tract in humans and animals, (2) neonatal meningitis *E. coli* (NMEC), which is responsible for meningitis and sepsis-related infections in infants, (3) septicemic *E. coli* (SEPEC), isolated from humans and animals with septicemia cases of various origins and (4) avian pathogenic *E. coli* (APEC), responsible for colibacillosis in chicken (Pitout, 2012). New animal pathogenic subgroups have been proposed as: mammary pathogenic *E. coli* (MPEC) and endometrial pathogenic *E. coli* (EnPEC) (Stromberg et al., 2017).

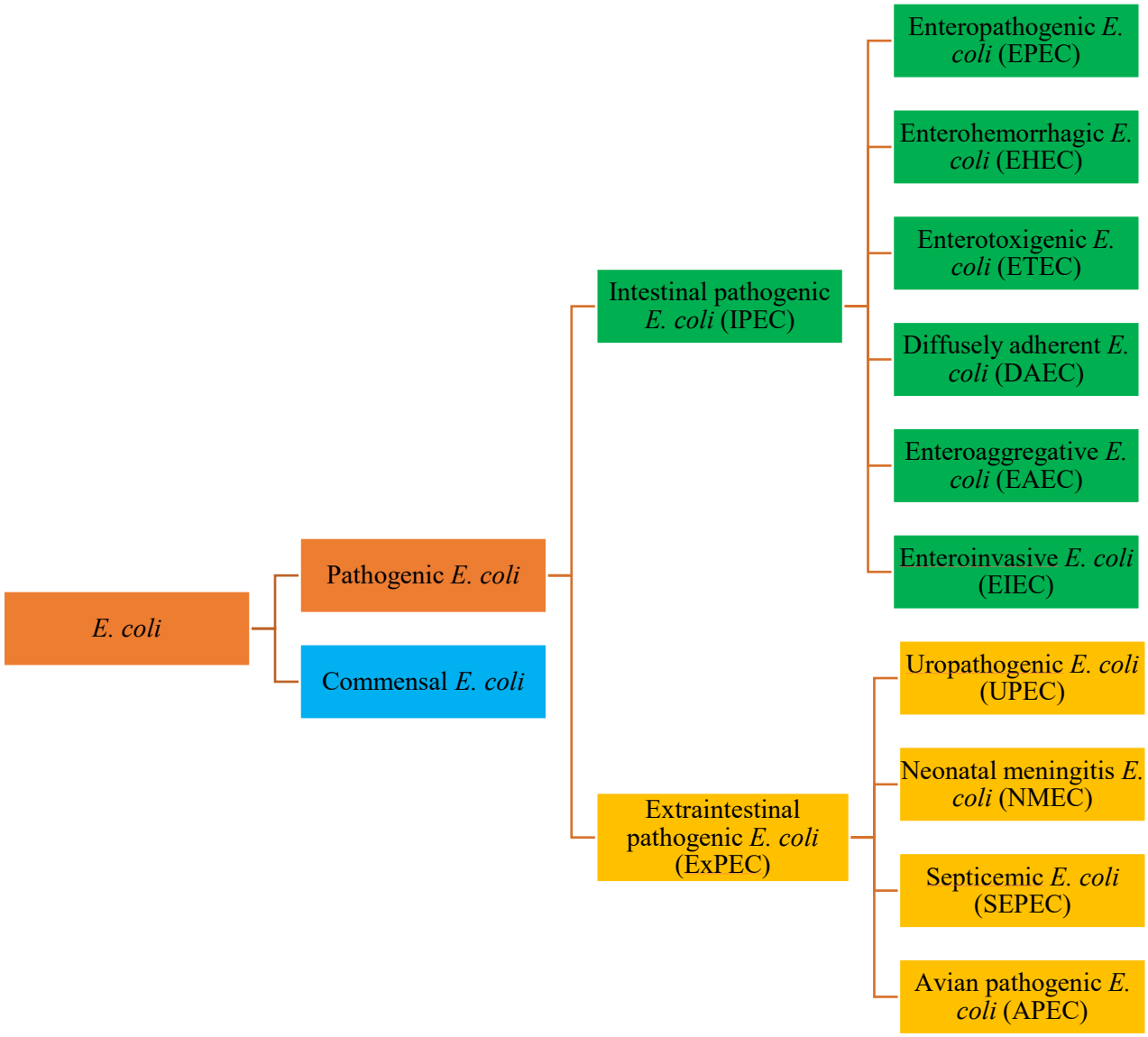

Figure 1: Pathotype classification of pathogenic intestinal and extraintestinal pathogenic *Escherichia coli*.

2.4 Source, transmission and clinical signs of Extraintestinal pathogenic *Escherichia coli*

2.4.1 In animals

Extraintestinal pathogenic *E. coli* colonizes the gastrointestinal tract of various animals and avian species, acting as a persistent reservoir for extraintestinal infections (Nedeljković et al., 2021). However, chickens represent a major source of ExPEC transmission to humans globally (Mitchell et al., 2015). In farms, animals get infected with ExPEC through their interaction with contaminated feed, soil drinking water and through animal handlers (Massella et al., 2021; Meena et al., 2021). Infected animals begin shedding the organisms with their feces as early as 14 weeks of age, contaminating the environment. In chickens, infection occurs either vertically (from parent to offspring) or horizontally in the hatchery environment through contact with contaminated surfaces, feed, water and air (Kabir, 2010).

Chickens can be contaminated with ExPEC at various points within the poultry production system (Stromberg et al., 2017). Hatchery and processing lines are a major reservoir of ExPEC (Fertner et al., 2011). Fecal contamination of eggs may cause yolk sac infection, and the pathogen can also penetrate the eggshell and spread to the chicks during the hatching process (Cox et al., 2012). This is known as egg infection, which operates as a vertical infection and infects a significant proportion of chicks in a manner that is comparable to horizontal transmission (Pande et al., 2016). Figure 2 illustrates the vertical and horizontal transmission routes and mechanisms of APEC in poultry. The transmission of APEC occurs both vertically from broiler breeders to chicks and horizontally among flock members via contaminated fluids, aerosols, feathers, and feces (Joseph et al., 2023a). Infected eggs can affect embryos, while non-infected chicks may get infected through contaminated direct contact (Christensen et al., 2021). Conditions during hatching and transport promote the spread of this highly transmissible pathogen, with infected birds potentially carrying the bacteria asymptomatically throughout the production period (Kaboudi & Jbenyeni, 2019).

Chicken-to-chicken pecking or inhaling infected fecal dust is also another way that ExPEC transmission can cause serious illness or death in chickens (Gomes et al., 2016). In addition, ExPEC transmission in chickens may lead to an increase in colonized birds, potentially increasing the recurrence of ExPEC transmission to poultry products (Stromberg et al., 2017).

Furthermore, important factors that increase the risks of ExPEC introduction and transmission are transportation of chicks and poor hygiene in farms (Swelum et al., 2021).

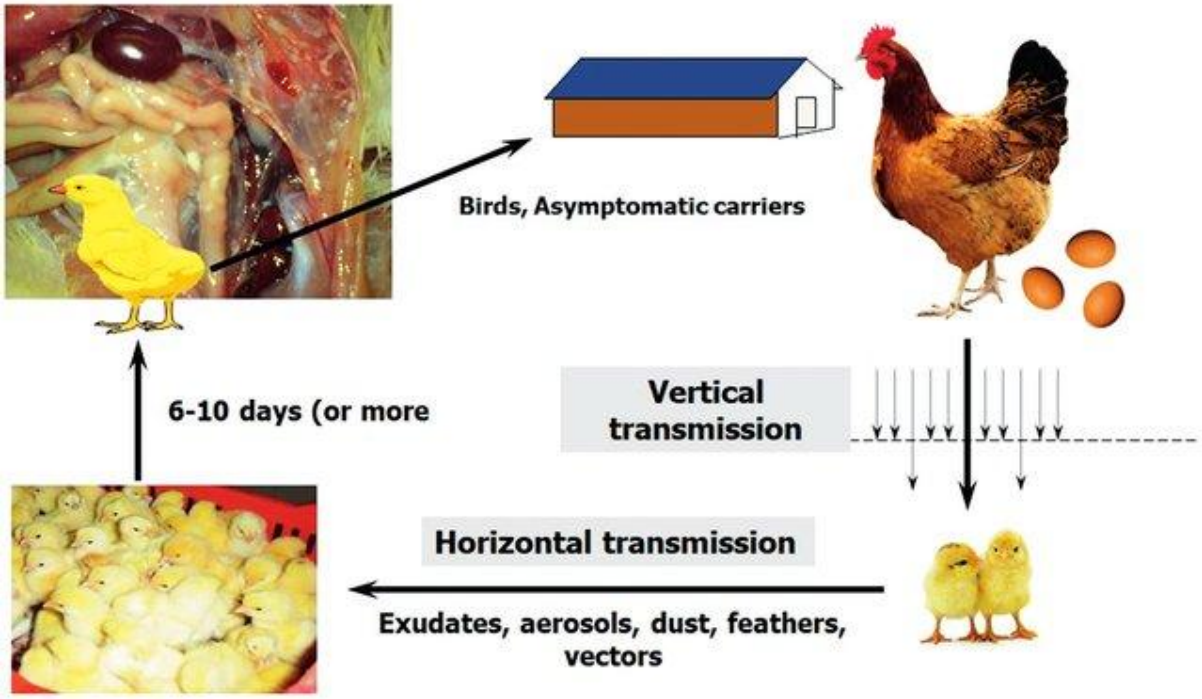

Figure 2: Vertical and horizontal transmission of ExPEC in chicken (Kaboudi & Jbenyeni, 2019).

Avian colibacillosis is a term used to describe several systemic diseases caused by ExPEC particularly by APEC strains in chickens (Nolan et al., 2013). A lifelong exposure to APEC is possible in chickens at all ages, although the most susceptible age range for broiler chicks is between 4 and 6 weeks (Kathayat et al., 2021). The clinical outcome of an APEC infection in birds is influenced by the bacterial strain, the host, the route of infection, and environmental factors that predispose the birds to the infection, even though the environment and the intestines act as reservoirs for the infection (Mellata, 2013).

Avian Pathogenic *E. coli* infection can cause acute septicemia, sub-acute fibrinous diseases of internal organs (pericarditis, airsacculitis, salpingitis, and peritonitis) (Kathayat et al., 2021). The buildup of fibrin and pus is indicative of pericarditis, an inflammation of the pericardium, which usually occurs when APEC moves from the respiratory system or through the bloodstream to the heart and is frequently linked to septicaemia (Timur et al., 2026). Airsacculitis - inflammation of the air sacs in poultry lungs, caused by APEC infection, leads

to inflammation and accumulation of pus in the air sacs, hindering gas exchange and causing respiratory distress (Kamal et al., 2025). Salpingitis refers to the inflammation of the fallopian tube in chickens, resulting in reduced egg production and the occurrence of abnormal eggs (Khairullah et al., 2024). Peritonitis the inflammation of the peritoneum, the serous membrane that covers the abdominal organs and lines the abdominal cavity (Igbokwe et al., 2025). Retained contaminated yolk causes omphalitis in new-born chicks of up to three weeks of age (Swelum et al., 2021). Necrotic dermatitis, also known as cellulitis, causes a persistent infection of the subcutis on the thighs and belly and this afflict broilers (Gornatti-Churria et al., 2018). Oedema of the cranial and periorbital skin is a symptom of Swollen Head Syndrome, a condition that mostly affects the broilers (Kabir, 2010). Layers and broilers are susceptible to acute or chronic salpingitis (Kabir, 2010). As a result, once the yolk material has been deposited in the peritoneal cavity, salpingitis in layers may cause egg peritonitis, which is the loss of the capacity for chickens to lay eggs (Srinivasan et al., 2013).

Every age group experiences airsacculitis, inhaling dust tainted with feces causing chronic respiratory disease (Barger, 2019). Chickens of all ages can get septicemia, primarily seen in broilers (Hernández, 2014). The most prevalent type of colibacillosis is polyserositis, which results in illnesses primarily characterised by symptoms such as fever, depression, greenish or yellowish droppings, and internal organ abnormalities. (Kabir, 2010). In addition, colibacillosis can result in panophthalmitis, enteritis, peritonitis, orchitis, osteomyelitis/synovitis (including Turkey Osteomyelitis Complex), coligranuloma (Hjarre's disease), and venereal colibacillosis (Nolan et al., 2013).

2.4.2 In humans

Several non-human reservoirs, such as animals, sewage, water, soil and other environmental sources, are potential sources for the *E. coli* strains that cause extraintestinal infections in humans (Bélanger et al., 2011). It is widely acknowledged that there are several ways by which ExPEC spread from animals to humans, including eating or handling contaminated meat and direct contact with an infected animal (Meena et al., 2023). Contaminated water sources, fruits and vegetables contaminated with animal or human waste containing ExPEC can also be a source of human infection (Frömmel et al., 2013).

Extraintestinal pathogenic *E. coli* strains are the cause of a wide range of invasive human illnesses (Smith & Fratamico 2017). Extraintestinal pathogenic *E. coli* has the capacity to invade bloodstream, several tissues (e.g. urinary tract, kidney, lungs (respiratory tract), bloodstream and abdominal cavity) and result in infecting people at different age groups. Extraintestinal pathogenic *E. coli* is the most prominent contributor to neonatal meningitis and the primary cause of bacteremia, which mainly affects the elderly (Mellata, 2013). Extraintestinal pathogenic *E. coli* often causes prostatitis (prostate gland), peritonitis (peritoneum-abdominal cavity lining), and pneumonia (lungs) (Poolman & Wacker, 2016). In Africa, neonatal meningitis is a primary cause of both morbidity and mortality with fatality cases ranging from 5 to 25%, and 25 to 50% of survivor's experience neurologic disorders (Barichello et al., 2023).

Extraintestinal pathogenic *E. coli* strains is a major cause of UTIs in young and immunocompromised women (Mellata, 2013). Approximately 80% of all human UTIs worldwide are thought to be caused by UPEC strains, affecting an estimated 130-175 million people annually (Bélanger et al., 2011). Urinary tract infections predominantly affect women than men, women are expected to experience one UTI during their lifetime. Women usually experience recurrence in about 25% within six to twelve months (Mellata, 2013). Pregnancy heightens the risk of infection due to hormonal changes, ureteral dilation, and urinary stasis, resulting in UTIs in 50–60% of cases. Postmenopausal women face increased susceptibility due to reduced estrogen and subsequent vaginal atrophy, which allows UPEC colonization (Czajkowski et al., 2021). Additionally, High glucose levels in urine facilitate bacterial growth, and diabetes can impair the immune system, raising infection risk. Uncontrolled diabetes further weakens immunity and increases susceptibility, while catheterized patients, especially in healthcare settings, see over one million catheter-associated UTIs annually (Werneburg, 2022).

Extraintestinal pathogenic *E. coli* strains is a leading cause of sepsis, especially in patients with weakened immune systems. it is the prominent cause of sepsis in hospitals, and infants, particularly newborns. ExPEC strain (SEPEC), often causing sepsis, typically originate from urinary tract infections UTIs. Extraintestinal Pathogenic *E. coli* accounts for about 30% of sepsis cases, especially among the elderly, and is also linked to specific diseases like nosocomial pneumonia and surgical site infections (Bélanger et al., 2011; Mellata, 2013; Wasiński, 2019).

2.5 Economic impact of Extraintestinal pathogenic *Escherichia coli* in the chicken industry

Chicken farming is one of the most significant industries in SA (Kathayat et al., 2021). The chicken industry is the largest sector of the South African agricultural sub-sector contributing to 16% of the domestic product (SAPA, 2021). Majority of global economic losses from chicken industry are attributed to infections caused by APEC, a subset of ExPEC (Mellata, 2013), despite providing a substantial contribution to the nation's gross domestic products, the poultry industry has challenges due to the burden of diseases that causes economic losses (Joseph et al., 2023). The primary sources of economic losses are the mortality rate, which reduces egg production, the disposal of carcasses, and reduced production that affects chicken flocks (Grace et al., 2024).

Infections with *E. coli* result in mortality rates of 1–10% in chickens (Swelum et al., 2021), with significantly higher rates in broilers (Kemmett, 2013). An estimation of $40 million is lost to the United States industry each year as a result of ExPEC. Studies show that 36-43% of processed broilers and carcasses having consistent lesions related to *E. coli* sepsis on occasion, and 54% of these mortality incidents in egg layers are linked to *E. coli* (Kathayat et al., 2021). Therefore, maintaining food safety management is essential for minimizing the burden of foodborne illnesses and improving consumer safety (Flynn et al., 2019).

2.6 Pathogenesis of Extraintestinal pathogenic *Escherichia coli*

2.6.1 Uropathogenic *Escherichia coli*

The pathogenesis of UPEC during UTIs infection begins in an ascending order including the following steps: (firstly) colonization occurs in the periurethral and vaginal regions alongside with colonization of the urethra; (secondly) ascent into the bladder lumen and develop into planktonic cells in urine; (thirdly) interaction with the bladder epithelium defence system and epithelial cells line in the lumen of the bladder breaking into the host cell cytosol and rapidly multiply; (fourthly) forming intracellular biofilm; (fifthly) The bladder's Intracellular Bacterial Communities facilitate invasion (bladder cells with UPEC exfoliate, clearing bacteria with urine flow, but expose immature bladder epithelial cells, making them more susceptible to infection) and replication (actin-bound bacteria, which are less susceptible to antibiotics and

less immunogenic, may serve as reservoirs for recurrent UTIs due to their restricted replication), while quiescent intracellular reservoirs form and reside in the underlying urothelium. (sixthly) The condition can lead to kidney colonization, host tissue damage, and an increased risk of bacteremia/septicemia (Wiles et al., 2008; Terlizzi et al., 2017) (figure 3). Numerous virulence factors are utilised by UPEC to colonise the bladder, and as a result, they serve a role in the pathogenesis (Karam et al., 2019).

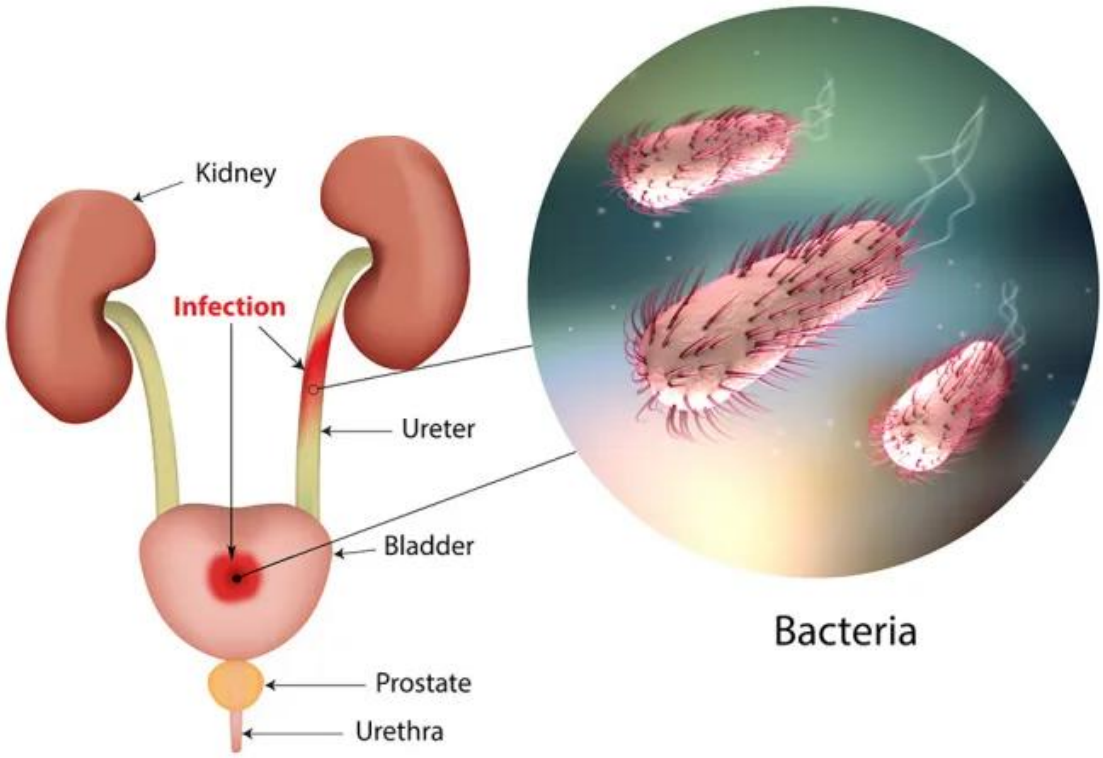

Figure 3: Overview of UPEC infection beginning in an ascending manner from the urinary tract through the urethra moving up till the kidney causing severe infections that damages the kidney (Terlizzi et al., 2017a)

2.6.2 Neonatal meningitis-causing *Escherichia coli*

Neonatal meningitis-causing *E. coli* enters the gastrointestinal tract and then the bloodstream, bypassing the blood-brain barrier (BBB) and entering the central nervous system (CNS) figure 4 (Herold et al., 2019). To enter the CNS, they require high bacteremia levels, mediated by the antiphagocyte capsule and outer membrane protein A (*OmpA*) (Nicholson et al., 2016). Ideally through replication and evasion of host defences, *E. coli* can enter the bloodstream and proliferate. The capsule, such as K1 in *E. coli*, enhances survival by inhibiting phagocytosis. *OmpA* aids in *E. coli* adhesion and invasion by interacting with host cells. Once within the

bloodstream, *E. coli* may lead to meningitis or other central nervous system infections by binding to brain microvascular endothelial cells using specific adhesins (Sora et al., 2021; Yang et al., 2021).

Moreover, NMEC replication in immune cells may play a role in achieving the required bacteremia. Furthermore, crossing of human brain microvascular endothelial cells by NMEC is preceded by type 1 fimbriae and *OmpA*-mediated attachment to the BBB (Nielsen et al., 2020), and invasion itself is mediated by the Ibe protein, FimH (type 1 fimbrial apical adhesin), *OmpA* and cytotoxic necrosis factor1 is mediated (Nicholson et al., 2016). The K1 capsule may also contribute to invasion by preventing lysosorrmal fusion and allowing viable bacteria to enter her CNS (Herold et al., 2019). Additionally, in animal models, plasmids are linked to an increase in NMEC virulence and produce cross-species and cross-pathogenic group gains of virulence function (Nicholson et al., 2016). It has also been observed that NMEC and UPEC tend to have similarities in terms of their pathogenesis since they share some of the critical virulence genes (Kathayat et al., 2021; Nicholson et al., 2016).

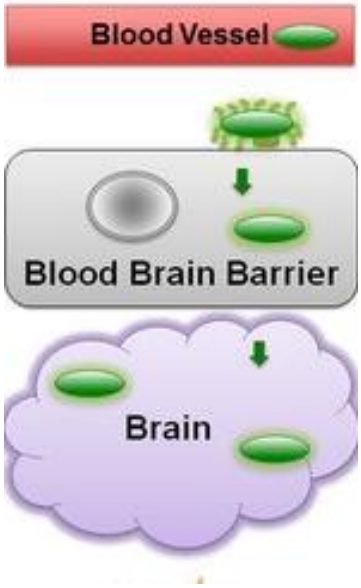

Figure 4: NMEC enters the bloodstream and crosses the blood-brain barrier, leading to inflammation and infection in the brain and spinal cord (Breland et al., 2017).

2.6.3 Septicemic *Escherichia coli*

The first stage of an infection is the attachment of Septicemic *E. coli* to host cells. The colonization of tissues and in some instances, cellular invasion followed by intracellular multiplication or persistence (Conceição et al., 2012). Invasion can lead to bacteremia, inflammation, and systemic inflammation (Huang et al., 2019). The adhesion process begins when surface elements (adhesins) attach to their ligands, host cell receptors, or extracellular matrix proteins (Conceição et al., 2012). Septicemic *E. coli* adherence to host tissues such as the kidney may be a key factor during sepsis by providing access to blood flow, once in the bloodstream, SEPEC strains must have a favourable genetic make-up to survive in the blood and thereby induce sepsis (Conceição et al., 2012). Excessive immune response can cause organ dysfunction and septic shock (Yang et al., 2025). Though SEPEC is an important pathotype implicated in sepsis that results in death of infants globally, it remains one of the less characterised pathotypes compared to UPEC and NMEC (Meena et al. 2022).

2.6.4 Avian Pathogenic *Escherichia coli*

In the presence of stresses, APEC enters the gastrointestinal and respiratory tracts through damaged tracheal and intestinal epithelium, reaching the bloodstream and internal organs (Filho et al., 2015). These features support the establishment of APEC infection in chickens by facilitating adhesion, invasion, evasion of host immunological responses, colonization, proliferation, and systemic dissemination figure 5 (Kathayat et al., 2021; Li et al., 2025). Other bacterial mechanisms contributing to APEC pathogenesis in chickens include type III and VI secretion systems, quorum sensing, transcriptional regulators, two-component systems, and metabolism-associated genes (Kathayat et al., 2021).

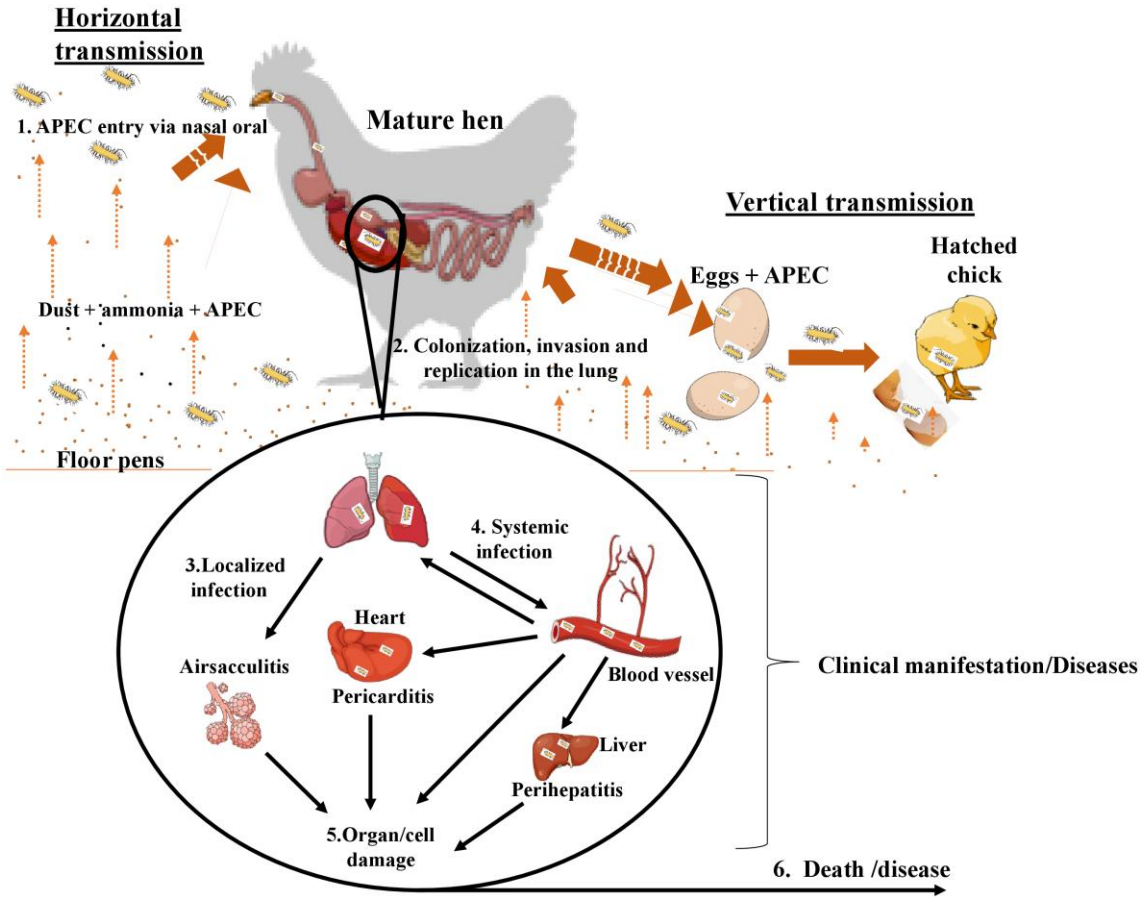

Figure 5: Detailed pathogenesis of APEC infection process in chickens (Waliaula et al., 2024)

2.7 Phylogenetic Grouping of *Escherichia coli*

Certain phylogenetic clusters are frequently isolated from different sources and rare in others. The phylogenic framework is imprinted on strains that live in a variety of settings, including soil, water, and the gastrointestinal tracts of birds and mammals (Geurtsen et al., 2022). *Escherichia coli* strains are classified into one of the progressive phylogenetic groupings (phylogroups; A, B1, B2, C, D, E, F and cryptic clade I) (Yu et al., 2021). According to epidemiological statistics by Clermont et al. (2000) the four phylogroups (A, B1, B2, and D) comprise of many *E. coli* strains. These groups are based on genotypic and phenotypic differences (Wu et al., 2021). The rest of the groups (C, E, F, and the cryptic clade I) are acknowledged as minor phylogroups (Geurtsen et al., 2022).

Characterizing the clonal structure within ExPEC phylogroups is essential for identifying subsets associated with specific features (e.g. antibiotic resistance profiles and virulence factor)

(Haghighatpanah et al., 2022). The combinations of genes using the Multi-Locus Sequence Typing (MLST) method can classify *E. coli* strains into phylogenetic groups, and the classification of Sequence Types allows identification of virulent ExPEC clones, (such as B2-ST131 and D-ST69) enhancing the understanding of their phylogenetic structure (Coura et al., 2015). Walk et al. (2009) discovered genetically diverse *E. coli* strains classified as cryptic Escherichia clades, divided into five groups (I to V). Clade I is closely related to *E. coli* and *E. fergusonii* strains, while clade V is the most divergent (Tourret & Denamur, 2016). These clades segregate strains based on virulence and resistance; commensal groups A and B1 can gain virulence through genetic transfer (Alfinete et al., 2022). A phylogenetic tree reveals two main clades: the B2 Clade, associated with high-virulence clones (ST73, ST95, ST131) causing UTIs, and the D Clade (ST69), associated with opportunistic infections and antimicrobial resistance. Key UPEC sequence types include ST131, ST1193, ST95, ST73, and ST69, significantly contributing to global UTIs and bloodstream infections (Walker et al., 2025). Phylogenetic trees from the *E. coli* reference collection reveal that group D diverged first, followed by sibling groups A and B1, which were separated later, but B2 is an ancient branch (Abdel Aziz et al., 2022). The B2 group, identifiable by at least nine subgroups (I to IX) defined by ST complexes under Achtman's MLST scheme, with examples including ST: 452, 144, 141, 131, 127, 95, 73, 14, and 12. Strain E2348/69 is mentioned as an additional B2 ST, belonging to the EPEC-1 group figure 6 (Tourret & Denamur, 2016).

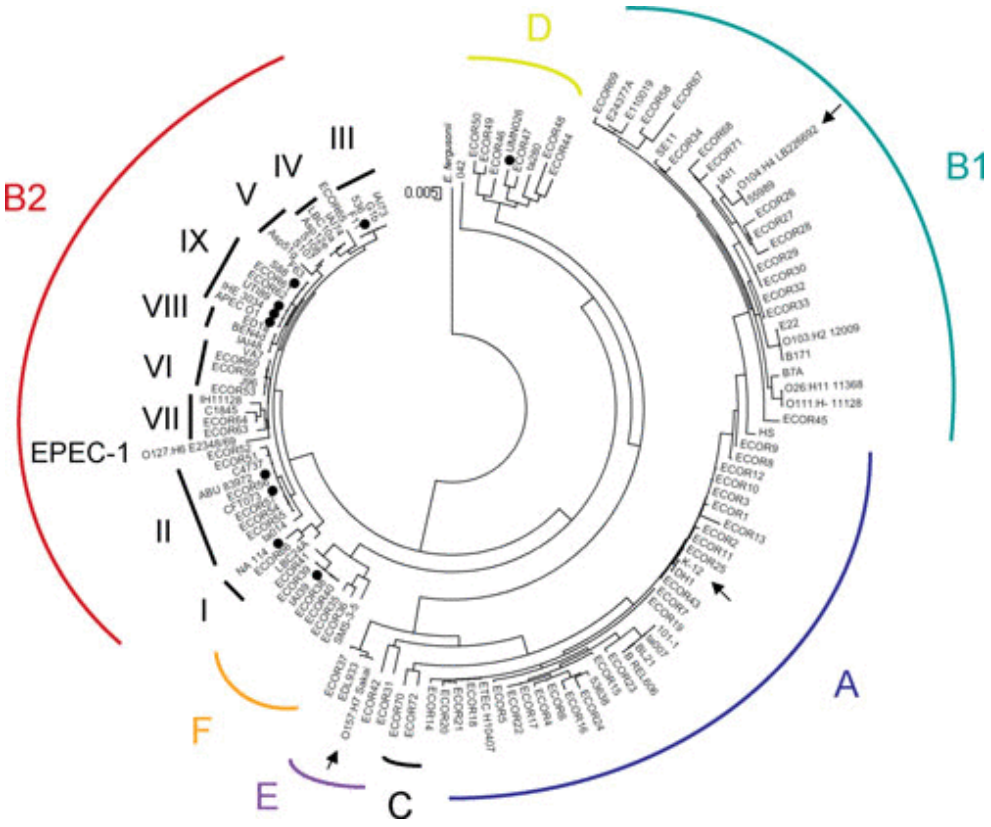

Figure 6: Phylogenetic history of 128 *E. coli* with the analysis rooted on *E. fergusonii* achieved using the Pasteur Institut MLST schema (Tourret & Denamur, 2016).

Human ExPEC strains often belong to group B2 which is more virulent; and group D which is less virulent (Čurová et al., 2020). Previous publications have demonstrated that commensal strains of *E. coli* belong to group A and B1, while pathogenic strains associated with extraintestinal infections predominantly belong to group B2 and, less likely to group D (Saralaya et al., 2015; Ramos et al., 2020; Geurtsen et al., 2022). Strains belonging to phylogroups B2 and D have elevated levels of virulence factors compared to strains belonging to phylogroups A and B1 (Saralaya et al., 2015). Saralaya et al. (2015) further shown that the commensal strains belonging to phylogroups A and B1 exhibit higher drug resistance yet with less virulence genes, while phylogroups B2 and D express more pathogenicity-associated islands and virulence factors.

The phylogroups exhibit distinct ecological niches, life histories, and characteristics like sugar exploitation, antibiotic resistance, and growth rate (Carlos et al., 2010). Several methods to determine *E. coli* phylogroups include PCR-based method, multi-locus sequence typing, ribotyping, and whole genome sequencing (WGS) (Alfinete et al., 2022). A PCR-based technique has been devised to characterise the phylogenetic approach to classify *E. coli* into

four various groups A, B1, B2, and D using the DNA fragment of *TspE4.C2* and the genetic markers *chuA* and *yjaA* using triplex PCR (Bozcal et al., 2018). Recently there was a development of categorizing *E. coli* strains, including an additional gene marker *arpA* subdividing them into eight phylogroups A, B1, B2, C, D, E, F, and clade I based on quadruplex PCR (El-Shaer et al., 2018). Horizontal genetic transfer pathways allow for the transmission of virulence groups between phylogroups, which may encourage the rare emergence of very virulent strains that belong to commensal phylogroups A or B1 (Sarowska et al., 2019). As a result, phylogenetic clustering of *E. coli* strains is useful for illustrating *E. coli* populations as well as the link between phylotypes and diseases the organism causes (El-Baz et al., 2022).

The composition of STs and clonal complexes (CCs) in *E. coli* is depicted in a minimum spanning tree (MST), highlighting CC 10 as the largest group, mainly consisting of non-pathogenic isolates, with few ExPEC strains (Figure 7). The right branch features primarily non-pathogenic *E. coli* CCs, while the left, designated the "ExPEC area," contains nine notables ExPEC CCs associated with extraintestinal virulence traits linked to phylogroup B2 (Köhler & Dobrindt, 2011). Despite the diversity and abundance within these CCs, clear distinctions between commensals, ExPEC, and subtypes such as UPEC, NMEC, and APEC are not evident. the *E. coli* MLST database illustrates population structure, showing color-coded pie charts that categorize isolates as confirmed and inferred ExPEC (dark and light red), confirmed IPEC (brown), inferred IPEC (light brown), non-pathogenic (green), and undefined (grey), with pie chart sizes reflecting strain counts in each ST and CC (Köhler & Dobrindt, 2011).

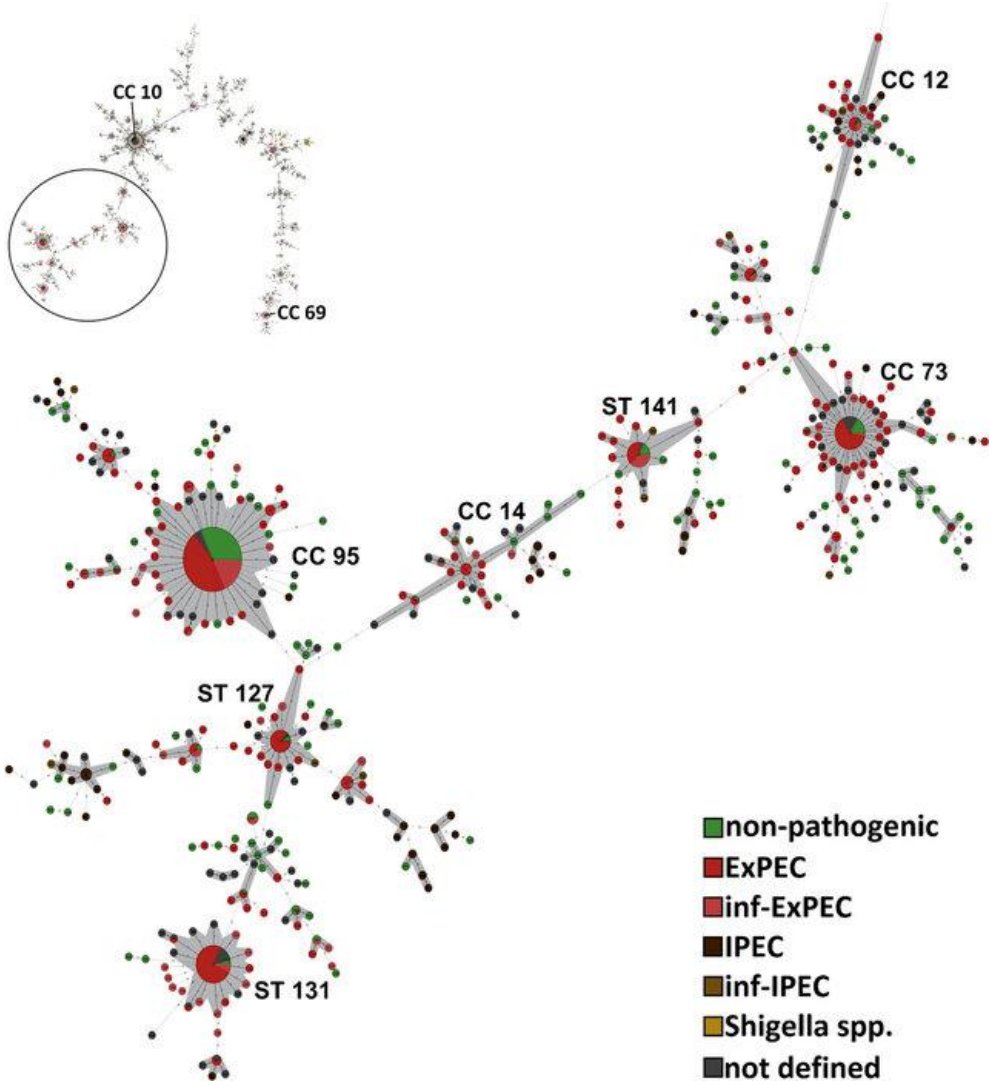

Figure 7: The phylogenetic background of ExPEC, IPEC, and non-pathogenic *E. coli* isolates through a minimum spanning tree created with Seqsphere software (Köhler & Dobrindt, 2011).

2.8 Virulence trait of Extraintestinal pathogenic *Escherichia coli*

The pathogenicity of extraintestinal *E. coli* is determined by the presence of virulence factors. These virulence factors include flagella, which facilitates bacterial motility; fimbriae, which facilitate adhesion and toxins facilitating ExPEC to survive inside the host by lysing immune cells, preventing phagocytosis, and obstructing leukocyte activity (Frömmel et al., 2013). Genes associated with virulence exhibit functions such as adhesion, invasion, attachment, iron uptake and toxin production (Pakbin et al., 2021). More than 50 virulence factors have been linked to ExPEC pathogenicity, however, a group of five genes (*afa/dra, iuc/iut, kpsMTII, pap* and *sfa*) allows the characterisation of strains that possess intrinsic virulence and can infect

healthy individuals extra-intestinally (Sarowska et al., 2019). The most important virulence factors of the ExPEC strain are described in detail in (Table 1) (Beceiro et al., 2013; Sarowska et al., 2019).

2.8.1 Flagella facilitating ExPEC adhesion

Flagella are curved filaments composed of flagellin that facilitate bacterial motility, they function as sensors, aiding ExPEC in navigating towards host organs, such as the kidneys through ureters (Akahoshi and Bevins, 2022). Flagella play a vital role in enabling bacteria, especially UPEC, to migrate towards host cells, facilitating tissue invasion and colonization in the urinary tract (Zhou et al., 2023). They are essential for initial attachment, biofilm development, and UPEC's ascent into the upper urinary tract (Terlizzi et al., 2017). Additionally, flagella contribute to bacterial adhesion and C of host cells, influencing virulence by regulating various factors and aiding in the migration and spread of infections, which may lead to ascending UTIs (Haiko & Westerlund-Wikström, 2013).

2.8.2 Fimbriae facilitating ExPEC adhesion

Fimbriae are hair-like projections based on the bacterial surfaces (thinner and shorter than flagella) that aid in adhesion of ExPEC (Elbaba, 2025). Extraintestinal pathogenic *E. coli* utilizes various fimbriae for binding to host cells: Type 1 fimbriae (*FimH*) attach to mannose-enriched receptors on bladder epithelium, enabling adherence against urine flow; P fimbriae target Galaα1-4Galβ-receptors in kidney cells, contributing to pyelonephritis; and S fimbriae bind sialic acid receptors, playing a role in CNS infections (Sora et al., 2021). This irreversible attachment is essential for colonization and invasion of deeper tissues (Sarowska et al., 2019).

Table 1: Extraintestinal pathogenic *Escherichia coli* virulence genes, functions, and encoded virulence factors

Description	Virulence genes	Function	Citation
Adhesins			Basavaraju & Gunashree. (2023); Sora et al. (2021); Sarowska et al. (2019); Meena et al. (2021)
Type 1 fimbriae	*Fm*	Colonization factors are critical in extraintestinal infections, due to their role in biofilm formation.	
Afimbrial adhesin	*Afa*	The nonfibrous adhesin interacts with the DAF receptor located on the epithelial cell surface, demonstrating hemagglutination capacity.	
Dr fimbriae	*Dra*	Binding to the DAF receptor on epithelial cells facilitates the internalization of bacteria in host cells.	
P fimbriae	*Pap*	Stimulating T lymphocytes to produce cytokines is important in extraintestinal infections, where colonization factors play a critical role.	
S fimbriae	*Sfa*	Adhering to intestinal epithelial cells, kidney cells, and lower urinary tract cells aids bacteria to effortlessly invade tissues.	
F1C fimbriae	*Foc*	Adhesion processes are crucial for the interaction of pathogens with renal epithelial cells and endothelial cells, especially in the bladder and kidneys.	
Iha	*Iha*	Adhesion of iron-regulated gene homologues.	
Mat	*mat*	Meningitis can be associated to temperature-regulated fimbriae.	
Curli fiber gene	*Crl, csg*	Facilitate formation of biofilm and enable pathogenicity.	
Antigen43	*agn43(fu)*	Autotransporter proteins are required for adhesion and biofilm development in bacteria.	
Invasins			
Ibe ABC	*ibeA,B,C*	Invasion of host cells.	
Iron uptake			
Aerobactin	*iuc,aer*	Iron (Fe2 +/3 +) acquisition in host system through siderophore.	
Iron repressible protein	*Irp*	Production of yersiniabactin.	
Salmochelin	*iroN*	Siderophore receptors facilitate the uptake of iron ions from the host body.	
ChuA, Hma	*chu, hma*	Permit the host system to utilize the iron derived from hemoglobin.	
SitABC	*sitA,B,C*	Transportation of iron and manganese.	

Protectins resistance			
Transfer protein	*traT*	Regulation of the complement activity through the classical pathway.	
Capsula antigens	*KpsMI-KpsMII*	Inhibition of the complement activity through the classical pathway.	
Outer membrane protein	*omp*	Facilitate intracellular survival while minimizing the body's defences.	
Increased serum survival	*Iss*	Phagocytosis-prevention factor.	
ColV, CvaC	*ColV, CvaC*	A factor which facilitates colonization easier.	
Toxins			
Serin protease autotransporter	*Pic*	Disrupts the cell membrane, breaks down mucins, and promotes colonization of the epithelium.	
Secreted autotransporter toxin	*Sat*	Proteolytic toxins induce cytotoxic effects leading to cell vacuolization.	
Vacuolating autotransporter toxin	*Vat*	Proteolytic toxin promotes vacuolization in host cells.	
Hemolysin A	*hlyA*	Cell lysis involves the formation of pores in the membranes of host cells.	
Cytotoxic necrotizing factor	*Cnf*	Contributes to cell necrosis.	
Cytolethal distending toxin	*Cdt*	Distending contributor to cytolethals.	

2.8.3 Toxins facilitating ExPEC survival

ExPEC secretes potent toxins to weaken host defence mechanism, crucial for survival and nutrient acquisition. Key mechanisms include α-hemolysin (*HlyA*), cytotoxic necrotizing factor 1 (*cnf1*), vacuolating autotransporter toxin (*vat*), secreted autotransporter toxin (*sat*), cytolethal distending factor (*cdt*), temperature-sensitive hemagglutinin (*tsh*) and protease involved in colonization (*pic*). *HlyA* lyses host cells, causing tissue damage, while *cnf1* modifies cell Rho GTPases to enhance bacterial invasion and prevent phagocytosis. *sat* targets bladder and kidney cells, linked to UTI pathogenesis, *cdt* induces DNA damage leading to cell cycle arrest, *tsh* contributes to respiratory infections and septicaemia and *pic* enhances colonization by degrading mucus and aids immune evasion (Sarowska et al., 2019; Pakbin et al., 2021; Sora et al., 2021).

Molecular definition of ExPEC isolates harbours at least two of five virulence factors: *afa*/*dra, iutA, kpsM II, foc/sfa, papA* and/or *papC* (Stromberg et al., 2017). Virulence factors play a crucial role in the pathogenicity of bacteria by aiding in adherence to host surfaces, invasion of host cells and tissues, evasion of host defence mechanisms, and triggering harmful inflammatory responses, ultimately leading to clinical diseases (Nicholson et al., 2016). This emphasizes the clear distinction that exists in the concept of ExPEC virulence factors between "virulence" and "fitness" and "colonization" factors (Beceiro et al., 2013).

2.8.4 Scientific description of virulent genes harbouring Extraintestinal pathogenic *Escherichia coli* infections factors

- *afa*/*dra:* identifies fimbrial adhesins in *E. coli*, crucial for attachment to host cells, particularly in UPEC, contributing to UTI and diarrhea (Whelan et al., 2023).
- *iutA:* encodes a receptor for the ferric-aerobactin complex, facilitating iron uptake, crucial for ExPEC survival in iron-limited environments (Landgraf et al., 2012).
- *kpsM II*: transports group 2 capsule polysaccharides, forming a protective capsule against host immune responses (Karbalaei et al., 2025).
- *foc/sfa:* operons code for specific fimbrial adhesins, S fimbriae and F1C fimbriae, vital for bacterial adhesion and virulence in UPEC and NMEC (Sarowska et al., 2019).
- *papA*: operons code for specific fimbrial adhesins, S fimbriae and F1C fimbriae, vital for bacterial adhesion and virulence in UPEC and NMEC (Wurpel et al., 2013).
- *papC:* assists in pilus assembly and surface expression, marking UPEC in urinary tract adherence (Werneburg and Thanassi, 2018).

2.8.5 Significance of virulent gene in Extraintestinal Pathogenic *Escherichia coli*

- *afa/dra* adhesin-positive *E. coli* are linked to kidney infections in pregnant women, increasing the risk of preterm labour or birth. The presence of *afa/dra* genes and virulence factors like P and S fimbriae raises the likelihood of bacterial translocation leading to sepsis. The *afa* gene cluster is vital for ExPEC virulence, enhancing adhesion and invasion, leading to severe infections. Additionally, the *dra* gene carry Dr-fimbriae that help bacteria attach to receptors on uroepithelial cells, contributing to chronic UTI's, particularly in pregnant women (Whelan et al., 2023).

- *csgA* gene carry curli fibres that enhance adhesion and biofilm formation, aiding ExPEC to colonize host surfaces and avoid the immune response (Abd El-Baky et al., 2020; García-García et al., 2025).
- *cvi/cva* gene operon in APEC, is an important virulence factor that carry Colicin V, it aids in eliminating competing bacteria, thereby enhancing colonization and survival in the host (Kathayat et al., 2021).
- *fimH* gene carry the fimH protein, crucial for binding to mannose receptors on host cells, facilitating type 1 fimbriae adhesion essential for UTIs. It is common in UPEC, aiding in receptor attachment, resistance to urine flow, and the formation of intracellular bacterial communities (IBCs) that contribute to persistent infections (Poole et al., 2017).
- *fyuA* gene in ExPEC carry the yersiniabactin siderophore receptor, crucial for iron acquisition and bacterial survival in iron-deficient environments. It is a significant virulence marker used to distinguish UPEC strains from commensal strains (Rezatofighi et al., 2021).
- *hlyA* gene carry α-hemolysin, a virulence factor in ExPEC (UPEC) that lyses host cells, causing tissue damage and inflammation in severe UTIs and sepsis. This cytotoxic pore-forming toxin, associated with urinary tract infections like cystitis and pyelonephritis, can damage erythrocytes and immune cells at high concentrations, while lower levels induce apoptosis and promote epithelial cell exfoliation, leading to renal complications and potential permanent damage (Xing et al., 2024).
- *ibeA* gene is a vital virulence factor in ExPEC, essential for its capacity to penetrate host barriers (BBB), particularly in NMEC and APEC, aiding in the invasion of brain microvascular endothelial cells and enhancing survival in macrophages (Sora et al., 2021).
- *iha* gene is a key virulence factor, functioning as a siderophore receptor for iron acquisition and an adhesin for bacterial colonization and pathogenesis. It is common in UPEC strains and is linked to the occurrence of UTI's (Krawczyk et al., 2025).
- *irp2* is responsible for the synthesis of yersiniabactin, an iron-binding siderophore that enables bacteria to extract iron from host proteins. It serves as a crucial virulence factor in ExPEC within the High Pathogenicity Island (HPI) and plays a significant role in iron acquisition, which is vital for bacterial survival and pathogenesis in iron-limited environments (Sora et al., 2021).

- *iss* gene in *E. coli* enhances survival in serum by diminishing the impact of the complement membrane attack complex through a protective polysaccharide capsule. This leads to increased virulence of ExPEC, contributing to extraintestinal infections (Biran et al., 2021).
- *iutA* gene carry a receptor for aerobactin that facilitates iron acquisition from the host, which is crucial for the survival, colonization, and infection of extraintestinal pathogens. It is frequently present in ExPEC strains linked to urinary tract infections, sepsis, and meningitis, making it a significant marker for identifying pathogenic and often multidrug-resistant strains (Ikeda et al., 2021).
- *kpsMII* is involved in capsular polysaccharides synthesis contributing to serum resistance and virulence, crucial for its survival in hosts, evasion of the immune system, and causing diseases like urinary tract infections and sepsis (Karbalaei et al., 2025).
- *mat* gene facilitates colonization, adhesion to host cells, and the development of biofilms. It plays a significant role in the pathogenesis of various infections such as NMEC (Basavaraju & Gunashree, 2023).
- *ompA* gene carry outer membrane protein A, which is a vital virulence factor involved in adhesion, invasion, and serum resistance. It maintains the structural integrity of the bacterial outer membrane and mediates interactions with the host, making it essential for the survival of ExPEC strains, particularly in blood and for crossing the BBB in NMEC (Nielsen et al., 2020).
- *papA* gene makes major structural subunit of P fimbriae, which aid in adherence to host tissues and are crucial in infections like pyelonephritis, sepsis, and UTIs (Fernández-Yáñez et al., 2024).
- *papC* is essential for P-fimbriae-mediated adhesion, allowing ExPEC to colonize host tissues, particularly for UPEC adherence to renal epithelium and, occasionally, bladder cells (Sarowska et al., 2019; García-García et al., 2025).
- *pic* carry an autotransporter protein that aids in the bacteria's survival, immune evasion, and tissue colonization within the host. *Pic* is present in ExPEC strains notably UPEC and APEC, where it contributes to extraintestinal infections (Desvaux et al., 2020).
- *sat* gene carry a toxic secretory autotransporter that acts as a class 1 Serine Protease Autotransporter, enhancing virulence in ExPEC strains. It promotes cytotoxicity and immune evasion, contributing to bacteremia and sepsis in UTI's and other extraintestinal diseases (Sora et al., 2021).

- *sfa/foc* gene cluster carry S fimbriae and F1C fimbriae, which facilitate the adhesin of bacteria to specific host cells, including urinary tract epithelia and the BBB (meningitis). This adhesin is crucial for colonization and subsequent infection (Sarowska et al., 2019).
- *sitA* is part of the *sitABCD* gene, carrying an iron and manganese transport system crucial for ExPEC virulence. This system supports the acquisition of essential metals, improving the bacteria's survival and pathogenicity in iron-limited environments (Sarowska et al., 2019).
- *traT* gene carry a surface protein that enhances serum resistance, allowing the bacteria to evade complement-mediated killing. This gene is linked to increased virulence and survival in extraintestinal infections (Sora et al., 2021).
- *vat* is a toxin causing vacuolation in host cells, contributing to tissue damage in the urinary tract for UPEC and the respiratory tract for APEC, and aiding bacterial survival during sepsis. It has been linked to increased virulence, respiratory infections, and cellulitis in broiler chickens (Nichols et al., 2016; Habouria et al., 2019).

2.9 Treatment of Extraintestinal pathogenic *Escherichia coli* infections

Antibiotics have been used for the treatment and management of bacterial infections including ExPEC benefiting both animals and humans (Mancuso et al., 2021). This is because they minimize patient morbidity and mortality in both human and animal patients (Manyi-Loh et al., 2018). Nevertheless, the excessive use of antibiotics in veterinary, medical, and agricultural settings lead to bacterial selection that are resistant and capable of resisting the antibiotic's action (Nwobodo et al., 2022). Since many developing nations lack access to high-quality treatments, health issues linked to antibiotic-resistant are more a result of limited options for treatment than disease pathology (Adefisoye & Okoh, 2016).

Consequently, infections continue to be a leading cause of mortality and morbidity. The ExPEC infections are frequently treated with cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (Wu et al. 2021). However, resistance to these antibiotics has been reported and adds complexity in effective therapy, which in turn raises elevated rates of hospitalization, morbidity, and death, which further contributes to increased healthcare burdens (Wu et al. 2021). For example, several surveillance studies across Europe, North America, and South

America indicate that 20 to 45% of ExPEC exhibited resistance to first-line antibiotics, such as cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (Pitout, 2012).

2.10 Significance of integrons in *Escherichia coli*

Escherichia coli, found in farm animals like swine, cattle, poultry, and aquatics, has been found to contain various classes of integrons (Kaushik et al., 2018). Integrons are genetic entities that recognize and capture mobile gene cassettes containing antibiotic resistance genes (Wu et al. 2021). An integron consists of a strong promoter (Pc), a gene for a site-specific recombination enzyme, a nucleotide sequence acting as a recombination site (attI), and an integrase from the family Tyrosine-Recombinase (intI) (Poirel et al., 2012; Nielsen et al., 2015). Despite the fact that integrons can be passed from one bacterium to another by transposons or plasmids, they are not regarded as mobile genetic elements (Ramatla et al., 2022). Integrons significantly contribute to the transmission of antibiotic resistance, particularly in Gram-negative pathogens (Kang et al., 2005). Integrons are widely recognized as the underlying cause of multiple resistance in Gram-negative bacteria more than in Gram-positive bacteria (Sabbagh et al., 2021). The transposons incorporate integron gene cassettes, creating mobile integrons that target plasmid res sites. This mechanism facilitates the development of AMRs in humans through horizontal gene transfer of resistant genes among various bacterial species (Figure 8) (Bhat et al., 2023).

Integrons are categorised into five classes according to the amino acid identity of the integrase gene; three of these classes (1, 2 and 3) have been linked to the distribution of resistance genes in *Enterobacteriaceae* (Poirel et al., 2012; Ahumada-Santos et al., 2020). Class 1 and 2 integrons are commonly found in *Enterobacteriaceae* bacteria, including *E. coli*, which often carries integrons with trimethoprim resistance genes (Racewicz et al., 2022).

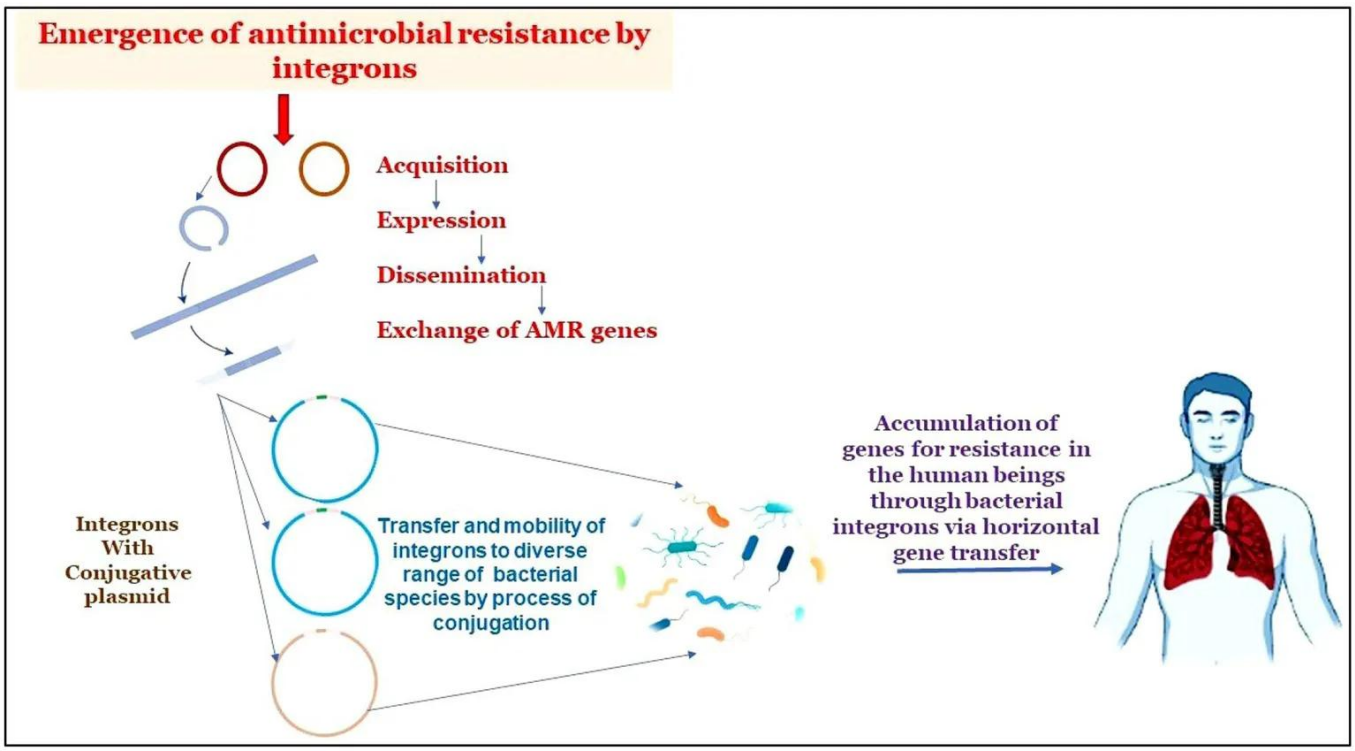

Figure 8: Depiction of horizontal transfer of integron from bacteria to human (Bhat et al., 2023)

2.10.1 Class 1 integron

Class 1 integrons are commonly linked to animals, particularly farm animals, as they are often raised for economic purposes and are more frequently exposed to antimicrobial agents (Kaushik et al., 2018). Class 1 integrons are prevalent in clinical Gram-negative bacteria isolates with a range of 22 to 59% (Deng et al., 2015; Sabbagh et al., 2021). In previous studies, 54% of urinary *E. coli* isolates had integrons (Kang et al., 2005). Integrons belonging to class 1 have greater mobility, distribution, and abundance than class 2 and 3 integrons, making them essential for carrying several resistance gene cassettes and the spread of AMR genes (Bhat et al., 2023). Class 1 integrons facilitate DNA transfer between pathogenic and commensal bacteria, accumulating antibiotic resistance genes in humans consuming antibiotics (Bhat et al., 2023). Furthermore, class 1 integron intersect food chains and move among bacterial species (Gillings et al., 2008).

2.10.2 Class 2 integron

Of all the different classes of integrons in *Enterobacteriaceae*, class 1 and class 2 are the most common classes found in isolates that are clinically significant (Kaushik et al., 2018). Class 2 integrons are typically located on transposon Tn7 that carries the promoter Pc and its recombination site attI2 within these transposons (Sabbagh et al., 2021). Class 2 integrons are nonreplicative Tn7 transposons featuring three gene cassettes (*dfrA1, sat2, aadA1*) next to the *intI2* gene. Their structure resembles that of class 1 integrons, and there is a noted association between the class 2 integrase *intI2* and the class 1 integrase (Cocchi et al., 2007). Class 2 integrons are home to gene cassettes resistant to trimethoprim (*dfrA1*), streptothricin (*sat2*), spectinomycin and streptomycin (*aadA1*) (Ramírez et al., 2010; Sabbagh et al., 2021). The significant difference between *intI1* and *intI2* is caused by the end codons prematurely stopping the integrase gene in class 2 integrons leading to the deactivation of 178 amino acid protein synthesis (Deng et al., 2015). Class 2 integrons have been found in various pathogens, a second variant of the integron, *intI2*, have been recently recruited onto an IncP plasmid in Uropathogenic *E. coli* (Gillings, 2014). This gene enables recombination reactions and carries a trimethoprim, streptothricin and streptomycin/spectinomycin resistance gene (Deng et al., 2015).

2.10.3 Class 3 integron

Few reports mention class 3 integrons in *E. coli*, which co-occur with class 1 integron in multi-drug-resistant isolates (Kaushik et al., 2018). The class 3 integron platform was integrated by a Tn402 transposon in a reverse orientation compared to the class 1 capture event (Bhat et al., 2023). Class 3 integron occurrence and identification rate range from 0-10%, with high-level resistance observed in 587 Gram-negative bacteria, 0.7% harbouring it, and positive results are occasionally found in veterinary isolates (Deng et al., 2015). Class 3 integron's occurrence and identification rate has varied from 0% to 10%. A study found that 587 Gram-negative bacteria showed high levels of resistance to both ceftazidime and sulbactam-cefoperazone, with 0.7% of the isolates containing the class 3 integron and reports have shown that at least 7% of veterinary isolates have also tested positive for class3 integrase by DNA-DNA hybridisation (Deng et al., 2015; Othman & Abdulkaliq, 2024).

2.11 Methods of isolation and identification of *Escherichia coli*

Various methods are employed in laboratories to detect the presence of *E. coli* in food. These methods include plate dilution method, membrane filtration, molecular identification, isolation on solid media, fluorescence quenching, and the use of quartz-crystal microbalance-based sensors (Sun et al., 2022). Despite the great reliability and sensitivity of these techniques, most of them still have certain limitations (Rentschler et al., 2021).

2.11.1 Most Probable Number methods

The Most Probable Number (MPN) approach is used to identify and enumerate *E. coli* in food and water samples as a sign of fecal contamination or unsanitary processing (Erkmen, 2021). The MPN is a statistical serial dilution assays, in which the results are compared with the standard statistical tables (Batt & Tortorello, 2014). The MPN involves three or more sets of dilutions containing fermentative broth and sample (Doğan-Halkman et al., 2003). The presence of acid and gas signifies a positive result in the analysis of coliform. This is quantified by counting the positive tubes and comparing the results with statistical data (Garthright & Blodgett, 2003). The advantage of this method is that it allows for is easy interpretations of test results and it is highly effective for analysing heavily contaminated turbid samples which cannot be tested by membrane filtration, however, drawbacks in this method is that it is labour intensive, has a long incubation period (7-10 days) and the results are not very accurate and requires large volumes of media and glassware (Bird et al., 2020).

2.11.2 Conventional culture-dependent methods

Standard microbiological techniques are also used for the isolation and identification of *E. coli* in clinical veterinary specimen (Markey et al., 2013). The homogenised samples are cultured onto Blood Tryptose agar and MacConkey agar, any developed colonies on MacConkey agar (pink colonies) are selected and subjected to Gram stain and a variety of biochemical assays (including the synthesis of indole, methyl-red, Voges-Proskauer and citrate tests) are used to confirm the suspect isolates (Lupindu, 2017). Various biochemical test methods such as Analytical Profile Index (API) 20E or API 10S can also be used for the identification of *E. coli* strain (Ramatla et al., 2021). Despite their widespread use, this method has a limitation, which includes the length of time it takes to get results; it is laborious to technicians to perform the

test and has poor differentiation between pathogenic and non-pathogenic strains (Hameed et al., 2018). This method is comparatively affordable, trustworthy, and simple to use (Ito et al., 2019). Furthermore *E. coli* can persist in food samples in a viable but uncultivatable condition (Das et al., 2023).

2.11.3 Rapid enumeration methods

The most popular approach for enumerating *E. coli* from food samples is the 3M Petrifilm Rapid *E. coli* which provides results within 24hrs (Bird et al., 2020). The 3M Petrifilm utilizes triphenyltetrazolium chloride and a glucuronidase indicator, resulting in a blue precipitate around *E. coli* colonies (Bird et al., 2020). The plate is hydrated with the sample and gelling agent to solidify the media (Silbernagel et al., 2003). Gas is produced during the fermentation of lactose by *E. coli* (Schraft & Watterworth, 2005). The chromogenic substrate 5-bromo-4-chloro-3-indolyl- ß-D-glucuronic acid is used to detect *E. coli* colonies by producing blue colonies, which indicate the presence of ß-glucuronidase activity (ISO 16649-1:2018). Glucuronidase-negative bacteria form red colonies as a result of the reduction of triphenyltetrazolium chloride (Silbernagel et al., 2003). This procedure is quick and easy to perform. However, it does not allow for further identification of isolates, when necessary, usually *E. coli* produce beta-glucuronidase, but some pathogenic *E. coli* does not produce this enzyme, and some non-*E. coli* strains can produce beta-glucuronidase enzyme (Bird et al., 2020).

2.11.4 Molecular methods

Polymerase Chain Reaction (PCR) is the widely used molecular-based technique for the detection of foodborne bacterial pathogens including *E. coli* (Law et al., 2015). Polymerase Chain Reaction can amplify minimal amounts of microbial DNA present in a sample to a thousandfold, it can provide information on the spread of multiple copies and bacterial pathogens (Kalle et al., 2014). Nucleic acid-based methods operate by detecting specific DNA or ribonucleic acid (RNA) sequences in the target pathogen this can be done by the following methods: simple PCR, multiplex PCR (mPCR) and real-time PCR (qPCR) (Zhao et al., 2014).

2.11.4.1 Conventional polymerase chain reaction

Conventional PCR works by amplifying a specific target DNA sequence in a three-step cyclic process: denaturation, annealing, elongation (Asif et al., 2021). First, double-stranded target DNA is denatured into single-stranded DNA at elevated temperatures followed by two single-stranded synthetic oligonucleotides or specific primers (forward primer and a reverse primer) that are annealed to the DNA strand (Khehra et al., 2025). The polymerization process involves the extension of a primer complementary to single-stranded DNA using deoxyribonucleotides and a thermostable DNA polymerase (Law et al., 2015). The PCR amplification products are visualized as bands on an electrophoresis gel by staining with ethidium bromide (Lorenz, 2012). This technique allows rapid amplification of specific DNA segments but is sensitive to cross-contamination, which can lead to inconclusive results (false positive/negative), its susceptible errors leading to mutations in the fragment generated (Garibyan & Avashia, 2013).

2.11.4.2 Multiplex Polymerase Chain Reaction

Multiplex PCR operates on a same core principle as conventional PCR. In contrast to conventional PCR assays, which only utilize one set of specific primers, mPCR assays use multiple sets of specific primers (Huang et al. 2018). The effectiveness of mPCR is greatly affected by primer design, especially the requirement for primer sets to possess identical annealing temperatures (Van Stelten & Nightingale, 2014). Additionally, primer concentration is critical due to potential interactions that can create primer dimers, necessitating adjustments to ensure reliable PCR product synthesis (Zhao et al., 2014). The additional key components of an effective mPCR assay are the concentrations of the PCR buffer, the ratio of magnesium chloride to deoxynucleotides, the amount of DNA template, the cycle temperatures, and Taq DNA polymerase (Law et al., 2015). In the past, mPCR could only identify two or three pathogens, although now that mPCR has advanced, it can simultaneously detect five or more pathogens (Kralik & Ricchi, 2017). Advantages of using mPCR include more information with less sample, higher throughput, cost effective — fewer deoxynucleoside triphosphates (dNTPs), enzymes, and other consumables and less input material required (Tobias & Vutukuru, 2012). The following drawbacks are indisputable: self-inhibition among various primer sets; poor amplification efficiency and lack of uniform efficiency on various templates (Han, 2013). These limitations would prevent its widespread use and further development, particularly in the high-throughput genetically modified organism (GMO) detection fewer pipetting errors (Kralik & Ricchi, 2017).

2.11.4.3 Real-time Polymerase Chain Reaction

In contrast to simple PCR, real-time PCR does not require agarose gel electrophoresis for the detection of PCR results (Sora et al., 2021). This method tracks the synthesis of PCR products by using dual-labelled probes or intercalating dyes to detect fluorescent signals (Gunson et al., 2006). The amount of amplicon used affects the fluorescence intensity of the signals (Mackay et al., 2004). A heat cycler enables real-time PCR by illuminating samples with a beam of light at specific wavelengths and detecting the resulting fluorescence from the fluorophore (Kubista et al., 2006). The thermal cycler can quickly heat and cool samples, temperature changes are often repeated couple of times as part of the PCR process (Mackay et al., 2004). The amount of amplified product is represented by the fluorescence levels, which are measured at each cycle utilising TaqMan and SYBR® Green fluorescent (Valones et al., 2009). These cycles typically have three stages: the first, which allows the nucleic acids double chain to be separated; the second, which enables the binding of the primers with the DNA template; and the third, which enhances the DNA polymerase's polymerization process (Kubista et al., 2006). Additionally, in four-step PCR, the fluorescence is monitored (Rodríguez-Lázaro & Hernández, 2013). The main advantages of qPCR include its rapid and effective detection and measurement of specific DNA sequences across different samples (Kralik & Ricchi, 2017). The simultaneous amplification and viewing of newly produced DNA amplicons expedites the amplification process. Furthermore, the absence of additional sample processing after amplification makes qPCR safer by minimizing the risk of cross-contamination, in addition to providing a wide dynamic range for quantification (Artika et al., 2022; Kralik & Ricchi, 2017). The drawback in this technique is that test kits are often packaged as ready-to-use limits options for optimization; moreover, the extraction kits are not always affordable (Valasek & Repa, 2005).

2.12 Whole Genome Sequencing

Whole genome sequencing has transformed microbiology by enhancing the understanding of bacterial pathogens such as ExPEC. Whole genome sequencing provides detailed insights about ExPECs, enabling identification of genetic profile, virulence factors, antibiotic resistance and phylogenetic relationships (Chen et al., 2021). It aids in identifying transmission routes, outbreak sources, while enhancing understanding of disease severity and resistance patterns (Katiyar et al., 2025). It facilitates strain comparison, revealing diagnostic targets and providing

high-resolution views on the evolution and resistance profiles of strains, enhancing epidemiological investigations and outbreak management (Gomes et al., 2025). Additionally, it also effectively aligns genotype with phenotype, improving antimicrobial resistance surveillance and foodborne disease outbreak investigations, which is critical for preventing and treating bacterial diseases (Chen et al., 2018). This genomic analysis is essential for tracking multidrug-resistant lineages, improving antimicrobial resistance monitoring, and speeding up species identification, thereby supporting outbreak management and treatment approaches (David et al., 2025). While WGS can provide valuable genomic information the widespread implementation of WGS faces challenges including high costs, the need for specialized bioinformatics skills, insufficient computational resources in low-resource areas, and a lack of standardized data-sharing frameworks among relevant agencies (Gomes et al., 2025). Whole genome sequencing computational demands and time requirements restrict its application in clinical settings (Bagger et al., 2024; Brlek et al., 2024).

2.13 Conclusion

Chicken meat is a vehicle for *E. coli*, including ExPEC, which can cause infections outside the intestinal tract of mammals, including urinary tract, septecimia, and meningitis. Extraintestinal pathogenic *E. coli* can lead to substantial economic loss due to reduced poultry production, increased motility and the costs associated with treatment and control measures. The ExPEC pathotypes are influenced by virulent genes, allowing bacterial adhesion, invasion, and toxin production to colonize and cause disease outside the intestine. Various methods such as bacterial isolation, molecular identification and whole genome sequencing have been employed in laboratories to identify the presence of this bacterium. In SA, this bacterium has not been widely studied in animals as their reservoir, this raises a serious concern considering this strains ability to be resistant to antibiotics such as fluroquinolones. Additionally, considering food security, this bacterium can be difficult to treat causing a wide range of diseases while exhibiting resistance to multiple antibiotics. The surveillance of ExPEC is essential as it will aid in understanding chicken abattoir infection rates, guiding abattoir policies, determining antimicrobial resistant pattens and ultimately aiding in treatment decisions. This study will contribute immensely to closing the knowledge gap regarding ExPEC in food producing animals in SA.

CHAPTER 3: MATERIALS AND METHODS

3.1 Research design and study area

A cross-sectional study was conducted across the various geographical locations within Gauteng Province of SA to determine the prevalence, characteristics and antibiotics resistance of ExPEC in broiler chicken carcasses. The sampling areas encompassed a diverse range of settings including three metropolitan municipalities (City of Tshwane, City of Johannesburg, and Ekurhuleni) and two district municipalities (Sedibeng and West Rand) (Figure 9). These municipalities were selected to represent both urban and peri-urban environments, allowing for a comprehensive understanding of the prevalence and antibiotic resistance patterns of ExPEC in different parts of the province.

Gauteng is the smallest of SA's nine provinces by land area occupying only about 1.5% of the country's total surface (Ijatuyi et al., 2025). It is situated on the Highveld with approximate Global Positioning System coordinates of 26.2708° S, 28.1123° E. Despite its small size, it is a very densely populated province and home to over 25% of the national population (Oelofse et al., 2018). This province plays a significant role in the poultry industry, housing approximately 11.6% (53 smallholder broiler farms and 27 registered chicken abattoirs) of poultry facilities, South African Poultry Association (SAPA, 2021; SAPA 2023; SAPA 2025).

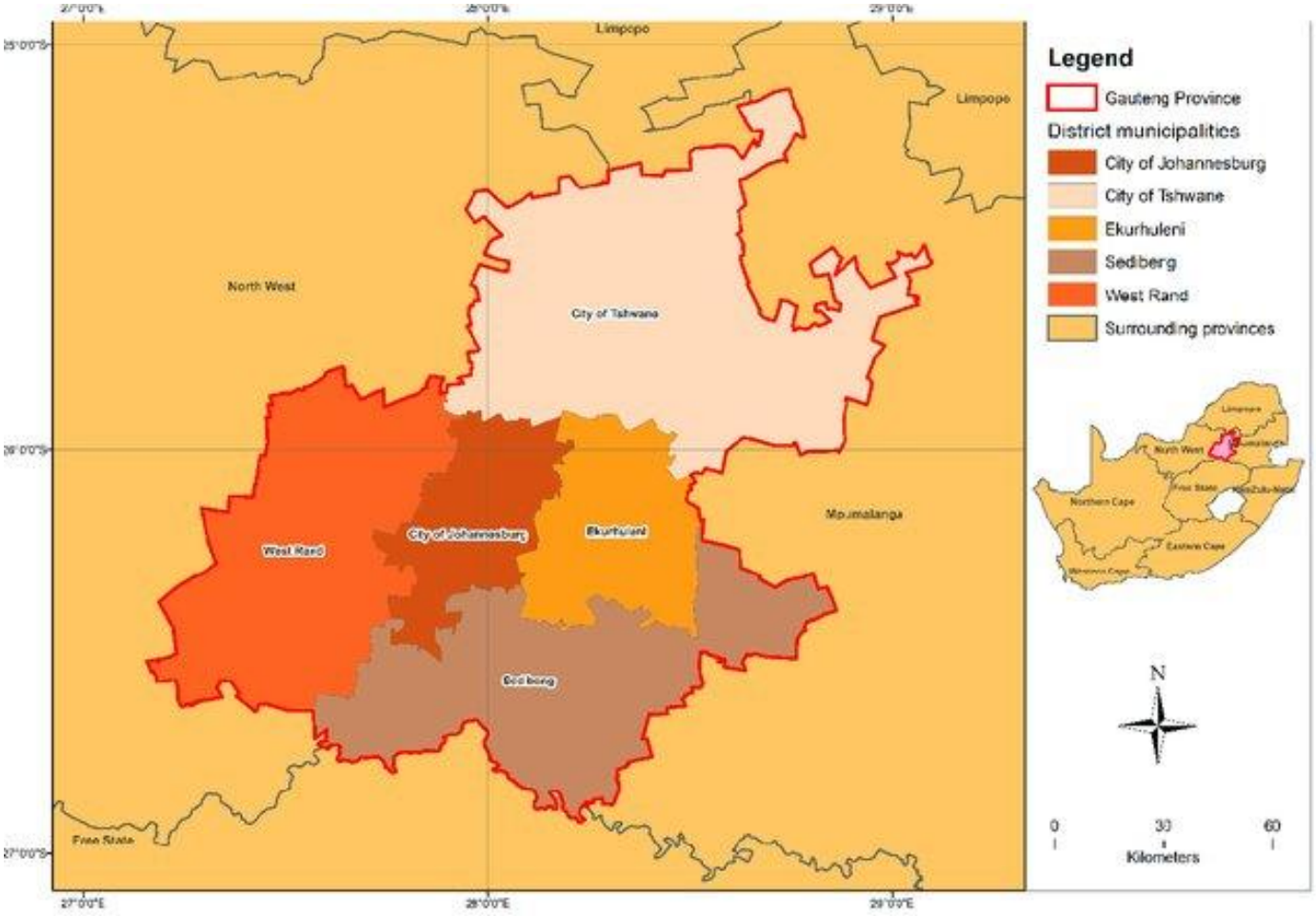

Figure 9: Map of Gauteng Province showing sampling collection areas (City of Tshwane, City of Johannesburg, Ekurhuleni, Sedibeng, and West Rand) (Source: Bidassey-Manilal et al., 2020).

3.2 Determination of sample size

Due to the absence of previously published data on prevalence of ExPEC in chicken carcasses in SA, sample size was estimated based on an assumed prevalence of 50%, with a 95% confidence interval and desired precision of 5% following the method described by Thrusfield, (2007) using formula 1.

Formula 1:……$n_0 = \frac{\{1.96^2 \times P_{exp} \times (1-P_{exp})\}}{d^2}$,

where P_{exp} is the expected occurrence and d is the desired precision. A P_{exp}value of 50% and a d value of 5% give

$$n_0 = \frac{3.84 \times 0.5 \times 0.5}{0.0025} = 384$$

Using the formula 1, this study's minimal sample size was determined to be 384 (Seakamela et al., 2022). The sample size was increased by 5% to account for potential sampling and laboratory errors hence final sample size was n = 404.

3.2.1 Selection and determination of the number of abattoirs

The study used stratified proportional sampling to determine the number of abattoirs per municipality. This approach divides the subgroups into stratums and uses a formula to select the appropriate number of individuals. Therefore, the Barreiro & Albandoz, (2001) study was adopted to determine the number of abattoirs from each stratum (municipality) using the following formula:

$$n_i = n.\frac{N_i}{N}$$

Where, n_i = required sample size for each stratum, n = sample size, N_i = population size for each stratum and N = size of the population (number of chicken abattoir). The sub-population (N_i) consists of City of Tshwane (n = 80), City of Johannesburg (n = 80), Ekurhuleni (n = 80), Sedibeng (n = 80) and West Rand (n = 84). The proportional sample size for each stratum was employed in this research by applying equation 2, as demonstrated in table 2.

Table 2: Summary of the sample stratum

Geographical location	Designation of slaughter	Number of abattoirs (n_i)	Number of samples (Ni)
City of Johannesburg (East Rand)	Abattoir (Formal)	4	40
	Home (Informal)	4	40
Ekurhuleni	Abattoir (Formal)	4	40
	Home (Informal)	4	40
Randfontein	Abattoir (Formal)	4	40
	Home (Informal)	4	40
City of Tshwane	Abattoir (Formal)	4	42
	Home (Informal)	4	38
West Rand	Abattoir (Formal)	4	44
	Home (Informal)	4	40
Total			404

3.3 Sample collection and transportation to the laboratory

A total of 404 chicken carcasses were purchased from chicken abattoirs (formal/informal). A minimum of 38 carcasses were obtained from each abattoir, and the samples were randomly selected during the purchasing process to ensure representativeness and minimize selection bias. Each carcass was placed in a sterile plastic bag and labelled with relevant sample information, including origin, date of collection, weight and location. The labelled carcasses were placed in cooler boxes with ice pads to maintain cold chain during transportation. All samples were promptly transported to the Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR) laboratory for ExPEC testing.

3.4 Sample processing and microbiological analysis

Upon arrival at the laboratory, chicken carcasses were aseptically cut into portions (chicken: breasts, thigh, drumstick, wings, neck and back) from various parts of the carcass. From each

carcass, 25g of meat was weighed and placed into a sterile, labelled Ziploc bag. The labelled samples were processed within 24 hours (hrs).

To determine the level of *E. coli* contamination in the chicken samples, the MPN method was employed using Brilliance Green Bile Broth (BG broth; Thermo Fisher Scientific, Hampshire, United Kingdom), following International Organisation for Standardisation (ISO) (ISO 7251:2005). Each 25g meat sample was aseptically transferred into 225 mL of Ringer's solution (Thermo Fisher Scientific, Hampshire, United Kingdom) and homogenized for 2 minutes (min) using a stomacher (WhiteSci, Cape Town, SA). Serial 10-fold dilutions were prepared using Ringer's solution. From three consecutive dilutions (10^{-1} to 10^{-3}), 1 mL aliquots were inoculated into triplicate tubes containing 9 mL BG broth with Durham tubes and incubated at 44 °C for 48 hrs (Labcon incubator, Johannesburg, SA). Following incubation, tubes were examined for turbidity and gas production, which indicated presumptive *E. coli* presence.

For biochemical confirmation, positive tubes (i.e., showing turbidity and gas) were streaked onto MacConkey agar and Eosin Methylene Blue (EMB) agar (Thermo Fisher Scientific, Hampshire, United Kingdom) (Moawad, 2021), and incubated at 37 °C for 24 hrs. On MacConkey agar, lactose-fermenting *E. coli* colonies appeared pink, while on EMB agar, characteristic colonies exhibited a green metallic sheen with a dark centre. Additionally, indole production was assessed by transferring BG broth cultures into tryptone water (Thermo Fisher Scientific, Hampshire, United Kingdom) and incubating at 44 °C for 24 hrs. Kovac's reagent was then added to detect indole production, a pink ring indicated a positive result, while no colour change indicated a negative result.

The number of positive tubes was used to estimate the MPN of *E. coli* per sample, calculated in accordance with ISO 16649-2:2003. Quality control was ensured using *E. coli* ATCC 25922 as the positive control and *Staphylococcus aureus (S. aureus)* ATCC 29213 as the negative control. All confirmed *E. coli* isolates were preserved by mixing cultures in a 1:1 ratio of Brain Heart Infusion (BHI) broth and 70% glycerol, then stored at −80 °C until needed for further analysis.

3.5 Phenotypic susceptibility testing of Extraintestinal pathogenic *Escherichia coli*

Antimicrobial susceptibility testing was performed using the Minimum Inhibitory Concentration (MIC) method via the broth micro-dilution technique, utilizing customized Gram-negative Sensititre™ 96-well microtiter plates (Thermo Fisher Scientific, Hampshire, United Kingdom). A bacterial suspension prepared to match the turbidity of a 0.5 McFarland standard, which is equivalent to 1.5×10^8 colony forming units (CFU/ml) was prepared by transferring colonies from Nutrient Agar into 5 mL of sterile distilled water. Following the manufacturer's instructions, 10 µL of the suspension (adjusted to 0.5 McFarland standard) was added to 9mL of Mueller-Hinton Broth (Thermo Fisher Scientific, Hampshire, United Kingdom) to obtain the final inoculum.

Fifty microliters (50 µL) of the final suspension were dispensed into each well of the 96-well Sensititre™ plate, which contained pre-coated antibiotics at various concentrations (Table 3). Inoculation into the plates was done within 30 min of preparing the final suspension. To confirm the sterility of the final suspension, a sample was simultaneously inoculated onto Nutrient Agar (Thermo Fisher Scientific, Waltham, USA). Plates were incubated at 37 °C for 18–24 hrs.

The MIC was recorded as the lowest concentration of the antibiotic that completely inhibited visible bacterial growth. Interpretation of susceptibility or resistance for ExPEC isolates was based on the clinical breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), specifically a breakpoint for each antibiotic (http://www.eucast.org; accessed 30 June 2023). Each test run included quality control strains to validate the assay: *E. coli* ATCC 25922 was used as a positive control, and *S. aureus* ATCC 29213 (Thermo Fisher Scientific, Waltham, USA) was used as a negative control.

Table 3: Classes, antibiotics and antibiotics concentration used in this study

Classes	Antibiotics	Concentration range (mg/L)
Cephalosporin	Cefoxitin (FOX)	32-0.5
Macrolides	Azithromycin (AZI)	16-0.12
Phenicols	Chloramphenicol (CHL)	32-2
Tetracycline	Tetracycline (TET)	32-4
Cephalosporin	Ceftriaxone (AXO)	64-0.25
Penicillins	Amoxicillin/ clavulanic acid (AUG2)	32/16-1/0.5
Fluoroquinolones	Ciprofloxacin (CIP)	4-0.015
Aminoglycoside	Gentamicin (GEN)	16-0.25
Cephalosporin	Ceftiofur (XNL)	8-0.12
Sulphonamide	Sulfisoxazole (FIS)	256-16
Sulphonamide	Trimethoprim/Sulfamethoxazole (SXT)	4/76-0.12/2.38
β-lactams	Ampicillin (AMP)	32-1

3.6 Molecular Analysis

3.6.1 Deoxyribonucleic acid template preparation

A subset of 63 previously confirmed *E. coli* isolates was included in this study to determine their classification as ExPEC. The DNA templates were prepared from these 63 isolates as well as those (404 isolates) obtained in the current study using the boiling method as previously described by Dashti et al., (2009). All isolates were thawed and cultured on MacConkey agar and Blood agar, followed by overnight incubation at 37 °C to allow for bacterial growth prior to DNA extraction. Pure colonies from overnight cultures were selected and sub-cultured on nutrient agar. Following incubation, colonies were harvested and suspended in 200µL sterile, DNase free water (Thermo Fisher Scientific, Waltham, USA) using 2ml Eppendorf tubes. The Eppendorf tubes were vortexed thoroughly, then heated in a dry heating block (Labnet International, USA) at 95°C for 15min to lyse the cells. The heated tubes were allowed to cool at room temperature for 10 min before being centrifuged (Sigma -Aldrich, Darmstadt, Germany) at 1300rpm for 5min. The resulting supernatant, that contained the crude DNA, was carefully transferred to a new sterile Eppendorf tube while the sediment was discarded. The

DNA templates were stored at -20°C for subsequent PCR amplification procedures. The quality and quantity of the DNA was assessed using the NanoDrop spectrophotometer.

3.6.2 Polymerase Chain Reaction confirmation of Extraintestinal pathogenic *Escherichia coli*

The ExPEC strains were confirmed using a multiplex PCR (mPCR) assay as described by Sukkua et al. (2017). This assay targets key virulence-associated genes commonly found in ExPEC strains specifically: *papA* (P fimbriae structural subunit) and/or *papC* (P fimbriae assembly), *sfa*/*foc* (S and F1C fimbriae subunits), *afa*/*dra* (DR antigen-binding adhesins), *kpsMT* II (group 2 capsular polysaccharide units) and *iutA* (aerobactin receptor) (Sukkua et al., 2017; Moawad, 2021). An ExPEC isolate is characterized by the presence of two or more virulence genes, specifically: *afa, iutA, kpsMTII, papA* and/or *papC*, and *sfaDE* (Johnson et al., 2003). The primer sequences used for amplification are listed in Table 4. The PCR amplification was performed in a 25 µL reaction mixture, which included 4 µL of DNA template, 11.5 µL of 2X GoTaq® Green Master Mix (Thermo Fisher Scientific, Waltham, USA), 3.5 µL of DNase-free water (Thermo Fisher Scientific, Waltham, USA), and 0.5 µL of each specific primer (10mM concentration - Inqaba Biotechnical Industries (Pty) Ltd, South Africa) listed in table 4. Thermocycling was carried out in a thermocycler (Bio-Rad, California, United States of America) with the following conditions: an initial denaturation at 95 °C for 2 min; 30 cycles of denaturation at 95 °C for 30 seconds (sec), annealing at 63 °C for 30 sec, and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 5 min.

Table 4: Oligonucleotide primer pairs used for identification of Extraintestinal pathogenic *Escherichia coli*

Target genes	Primer sequence (5'-3')	Product length (bp)	Reference
Afa	F: GCTGGGCAGCAAACTGATAACTCTC	750	(Sukkua et al., 2017)
	R: CATCAAGCTGTTTGTTCGTCCGCCG		
IutA	F: GGCTGGACATCATGGGAACTGG	300	
	R: CGTCGGGAACGGGTAGAATCG		
kpsMTII	F: GCGCATTTGCTGATACTGTTG	272	
	R: CATCCAGACGATAAGCATGAGCA		
papA	F: ATGGCAGTGGTGTCTTTTGGTG	720	
	R: CGTCCCACCATACGTGCTCTTC		
papC	F: GTGGCAGTATGAGTAATGACCGTTA	200	
	R: ATATCCTTTCTGCAGGGATGCAATA		
sfaDE	F: CTCCGGAGAACTGGGTGCATCTTAC	408	
	R: CGGAGGAGTAATTACAAACCTGGCA		

3.6.3 Gel preparation and deoxyribonucleic acid fragment observation

All PCR amplicons were separated using electrophoresis on a 2% agarose gel (Thermo Fisher Scientific, Waltham, USA) stained with 2 µL of ethidium bromide. A 100 bp molecular weight ladder was used as a size reference. Bands were visualized under ultraviolet (UV) illumination using a Syngene gel documentation system (Vacutec, Europe). Each PCR run included a positive control, consisting of an in-house sequenced *E. coli* strain harbouring the target genes, and a negative control using DNA-free water to validate the results.

3.6.4 Antibiotic resistance gene profiling of Extraintestinal pathogenic *Escherichia coli*

Multiplex PCR (mPCR) assays were performed to detect antimicrobial resistance genes across six major antibiotic classes: (1) Tetracyclines (*tetA*, *tetB*), (2) Trimethoprim (*dfrA1*, *dfrA5*, *dfrA7*), (3) Sulphonamides (*sul1*, *sul2*, *sul3*), (4) Phenicols (*cat1*, *floR*, *cmlA*), (5) β-lactams (*blaTEM*, *blaCMY-2*, *blaSHV*, *blaPSE*), and (6) Quinolones (*qnrA*, *qnrB*, *qnrS*). Presence of

these resistance genes was used to construct resistance profiles of each isolate. Polymerase Chain Reaction amplification was conducted in a 13 µL reaction volume comprising 2 µL of DNA template, 6 µL of Taq 2X Master Mix RED (Ampliquor, Denmark), 0.5 µL of each primer (10mM concentration - Inqaba Biotechnical Industries (Pty) Ltd, South Africa), and DNase-free water to make up the remaining volume. The volume of DNase-free water varied depending on the multiplex set: 3 µL for Tetracycline, 1 µL for β-lactam, and 2 µL for the other classes. Thermocycling was carried out using a thermal cycler (Germany) under the following conditions: initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at temperatures specific to each multiplex set (Table 5), and extension at 72 °C for 1 minute. A final extension step was performed at 72 °C for 10 min.

3.7 Detection of virulence genes

The detection of virulence genes was carried out in various mPCR as previously described by Frömmel et al. (2013). The following mPCR was performed in 25µl reaction mixture containing a template of 4µl DNA, 12.5µl mM Taq (Separation Scientific, Johannesburg, SA), 3.5µl DNase free water (LASEC, Pretoria, SA) and 0.5µl each primer (10mM concentration - Inqaba Biotechnical Industries (Pty) Ltd, South Africa). Various thermocycling conditions were carried out in a thermocycler (Bio-Rad, California, United States of America) as follows: mPCR 1, mPCR 3 and mPCR 4 conditions; initial denaturation at 94°C for 3 min, denaturation at 94°C for 30sec, annealing temperature at 52°C for 30 sec, and extension at 72°C for 1 minute cycling 40 times, with a final 5 min extension at 72°C, for mPCR 2; initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 sec, annealing temperature at 53°C for 30 sec, and extension at 72°C for 1 minute cycling 35 times, with a final 5 min extension at 72°C. Table 6 outlines the specific genes, primer sequences and the expected product size of target genes.

Table 5: Phenotypic oligonucleotide primers pairs resistance genes

Antibiotic group	Target genes	Nucleotide sequence (5'-3')	Product length (bp)	Annealing Temp(°C)	Reference
β -lactams	*blaTEM*	F TTAACTGGCGAACTACTTAC	247	55	Awosile et al. (2017)
		R GTCTATTTCGTTCATCCATA			
	blaCMY-2	F GACAGCCTCTTTCTCCACA	1000		
		R TGGACACGAAGGCTACGTA			
	blaSHV	F AGGATTGACTGCCTTTTTG	393		
		R ATTTGCTGATTTCGCTCG			
	blaPSE	F TGCTTCGCAACTATGACTAC	438		Ranjbar et al. (2020)
		R AGCCTGTGTTTGAGCTAGAT			
Trimethoprim	*DfrI*	F CGGTCGTAACACGTTCAAGT	220	55	Matayoshi et al. (2015)
		R CTGGGGATTTCAGGAAAGTA			
	DfrXII	F AAATTCCGGGTGAGCAGAAG	429		
		R CCCGTTGACGGAATGGTTAG			
	DfrXIII	F GCAGTCGCCCTAAAACAAAG	294		
		R GATACGTGTGACAGCGTTGA			
Sulphonamides	*sul1*	F CGGCGTGGGCTACCTGAACG	433	63	Pavelquesi et al. (2021)
		R GCCGATCGCGTGAAGTTCCG			
	sul2	F CGGCATCGTCAACATAACCT	293		
		R TGTGCGGATGAAGTCAGCTC			

	sul3	F CAACGGAAGTGGGCGTTGTGGA	244		
		R GCTGCACCAATTCGCTGAACG			
Quinolones	*QnrA*	F ATTTCTCACGCCAGGATTTG	516	53	Takaichi et al. (2022)
		R GATCGGCAAAGGTTAGGTCA			
	QnrB	F GATCGTGAAAGCCAGAAAGG	469		
		R ACGATGCCTG¬GTAGTTGTCC			
	QnrS	F ACGACATTCGTCAACTGCAA	417		
		R TAAATTGGCACCCTGTAGGC			
Chloramphenicol	*cat1*	F CTTGTCGCCTTGCGTATAAT	508	53	Odoch et al. (2018)
		R ATCCCAATGGCATCGTAAAG			
	Flo	F CTGAGGGTGTCGTCATCTAC	673		Chen et al. (2005)
		R GCTCCGACAATGCTGACTAT			
	CmlA	F CGCCACGGTGTTGTTGTTAT	394		Odoch et al. (2018)
		R GCGACCTGCGTAAATGTCAC			
Tetracycline	*TetA*	F GGCGGTCTTCTTCATCATGC	502	59	Pavelquesi et al. (2021)
		R CGGCAGGCAGAGCAAGTAGA			
	TetB	F CGCCCAGTGCTGTTGTTGTC	173		
		R CGCGTTGAGAAGCTGAGGTG			

Table 6: Primer sequences, amplicon sizes and PCR conditions used for virulence gene detection.

Multiplex PCR	Target genes	Nucleotide sequence (5'-3')	Product length (bp)	PCR conditions
*mPCR1	*afa-dra*	F TAAGGAAGTGAAGGAGCGTG	210	Initial denaturation at 94°C for 3 min, denaturation at 94°C for 30sec, annealing temperature at 52°C for 30 sec, and extension at 72°C for 1 minute cycling 40 times, with a final 5 min extension at 72°C
		R CCGCCCTGAAGAAGTATCAC		
	hlyA	F GTCCATTGCCGATAAGTTT	120	
		R ATAGCTCCTGTTTCTTTGTG		
	Pic	F ACTGGATCTTAAGGCTCAGG	168	
		R TTCCCCCACAGAGTGTTAC		
	sfa-foc	F CGGAGAACTGGGTGCATCTTA	189	
		R ACGCATGTGCTTCATCATG		
*mPCR2	*cvi-cva*	F CGCAGCATAGTTCCATGCT	172	
		R GCAATTTGTTGCAGGAGGA		
	iucD	F GTGTCAGAACGGAATTGTC	230	
		R GGGTATCTGTATCATGGCAAG		
	iss	F GGACAAGAGAAAACTGTTGATGC	129	
		R CAGCGGAGTATAGATGCCA		
	papC	F GCTCCATGGTCATATAGTTTCG	155	
		R TGATATCACGCAGTCAGTAGC		
	vat	F GTGTCAGAACGGAATTGTC	230	

		R	GGGTATCTGTATCATGGCAAG		
*mPCR3	*cnf1-cnf2*	F	CTTTACAATATTGACATGCTG	446	
		R	TCGTTATAAAATCAAACAGTG		
	csgA	F	GCTGCGTTACGATGGAAAGT	118	
		R	GATAACAGCGTATTTACGTGGG		
	mat	F	TATACGCTGGACTGAGTCGTG	275	
		R	GCCTGGAGTTTACTGAACCAA		
	Sat	F	CAACGGAAGTGGGCGTTGTGGA	220	
		R	GCTGCACCAATTCGCTGAACG		
#mPCR4	ibeA	F	TGGAACCCGCTCGTAATATAC	113	Initial denaturation at 94°C for 3 min, denaturation at 94°C for 30sec, annealing temperature at 53°C for 30 sec, and extension at 72°C for 1 minute cycling 35 times, with a final 5 min extension at 72°C
		R	AGTAGCTGCGCCTTCACG		
	iroN	F	ATCCTCTGGTCGCTAACTG	167	
		R	TTTTTAATATCCTCGCTGGTAA		
	ompA	F	GGTGTTGCCAGTAACCGG	177	
		R	GACGGTTCCGTAGTTGTTCTG		
	traT	F	GTGGTGCGATGAGCACAG	124	
		R	TCAGACGTGTTTTTGATCTGC		
	ibeA	F	TGGAACCCGCTCGTAATATAC	113	
		R	AGTAGCTGCGCCTTCACG		

***The PCR cycles for mPCR1, 2 & 3 was:** Initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 sec, annealing temperature at 52°C for 30 sec, and extension at 72°C for 1 minute cycling 40 times, with a final 5 min extension at 72°C, **# The PCR cycle for mPCR 4 was:** initial denaturation at 94°C for 3 min, denaturation at 94°C for 30sec, annealing temperature at 53°C for 30 sec, and extension at 72°C for 1 minute cycling 35 times, with a final 5 min extension at 72°C.

3.8 Phylogenetic subgrouping Extraintestinal pathogenic *Escherichia coli*

Phylogenetic grouping of *E. coli* isolates was conducted using singleplex PCR targeting three genetic markers: *chuA*, *yjaA*, and *TspE4.C2*, as described by Clermont et al. (2000) and modified by Frömmel et al. (2013). This method allows classification into the four major *E. coli* phylogenetic groups: A, B1, B2, and D, the groups are assigned based on presence or absence of target markers (figure 9). Primer sequences are presented in Table 7. The PCR reactions were performed in a total volume of 25 µL, comprising 4 µL of DNA template, 12.5 µL of Taq 2X Master Mix RED (Ampliquor, Denmark), 4.5 µL of DNase-free water (LASEC, Pretoria, SA), and 2 µL of the specific primers set (10mM concentration - Inqaba Biotechnical Industries (Pty) Ltd, South Africa). Thermal cycling conditions included an initial denaturation at 94°C for 3 min, followed by 33 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 5 min.

Table 7: Classification of phylogenetic grouping primer pairs

Target genes	Primer sequence (5'-3')	Product length (bp)
chuA	F: TGCCGCCAGTACCAAAGACA	121
	R: TTCAGCATTACTGTATGGCAGTG	
yjaA	F: CTGCCTTCAGTAACCAGCG	106
	R: AAAGAATGCCAGGTTGAACG	
TSPE4.C2	F: GAGTAATGTCGGGGCATTCA	90
	R: GAGACAGAAACGCGGGTAGA	

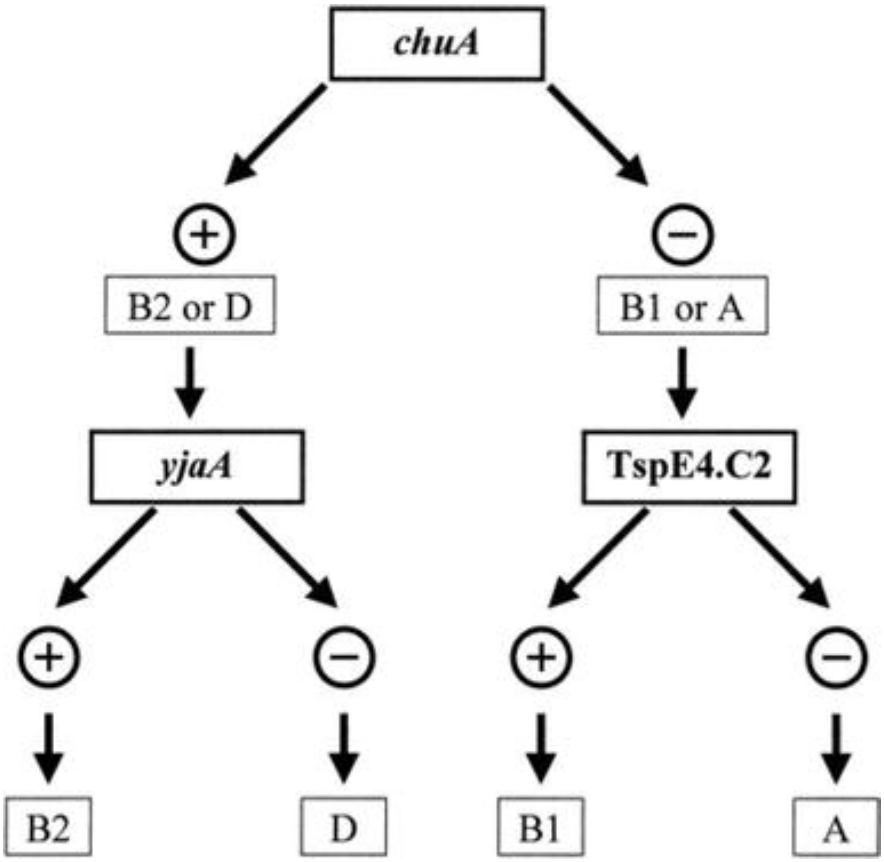

Figure 10: Decision trees employed to classify Extraintestinal Pathogenic *Escherichia coli* into phylogenetic groups (Clermont et al., 2000)

3.9 Detection of integron genes in Extraintestinal pathogenic *Escherichia coli* isolates

Confirmed ExPEC isolates were subjected to PCR screening to detect integron-encoded genes associated with antimicrobial resistance. Specifically, genes encoding class 1 (*intI1*), class 2 (*intI2*), and class 3 (*intI3*) integrons were targeted, as described by Ramatla et al. (2022). The following single-plex PCR was performed in 25 µl reaction mixture containing a template of 4 µl DNA, 12.5 µl mM Taq (Separation Scientific, Johannesburg, SA), 4.5 µl DNase free water (LASEC, Pretoria, SA) and 2 µl primer (10mM concentration - Inqaba Biotechnical Industries (Pty) Ltd, South Africa). The primer sequences and thermocycling conditions are provided in Table 8. The PCR reactions were carried out using Taq 2X Master Mix RED (Ampliquor, Denmark), as previously described by Ramatla et al. (2022). The reaction conditions were followed according to the specifications listed in Table 8.

Table 8: Primer sequences and PCR conditions for the detection of integron genes, *int1, intI2* and *intI3*

Target genes	Primer sequence (5'-3')	PCR Conditions	Product length (bp)
intI1	F: GCCTTGCTGTTCTTCTACGG R: GATGCCTGCTTGTTCTACGG	Initial denaturation 94°C for 5min Denaturation at 94°C for 30sec Annealing at 60°C for 30sec Extension at 72°C for 2min 35 cycles Final extension at 72°C for 5min	558
intI2	F: CACGGATATGCGACAAAAAGG R: TGTAGCAAACGAGTGACGAAATG	Initial denaturation at 94°C for 5min Denaturation at 94°C for 60sec Annealing at 60°C for 60sec Extension at 72°C for 2min 32 cycles Final extension 72°C for 10min	740
int3	F: GCCTCCGGCAGCGACTTTCAG R: ACGGATCTGCCAAACCTGACT	Initial denaturation at 94°C for 10min Denaturation at 94°C for 40sec Annealing at 59°C for 50sec Extension at 72°C for 55sec 35 cycles Final extension at 72°C for 10min	650

3.10 Statistical data analysis

Data was analysed using the Chi-square test (Ott & Longnecker, 2010) were performed using Frequency Procedure (PROC FREQ) of SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC, USA) to determine whether there were statistically significant differences or associations in the variables of each objective (Independent variables: region and/or designation of slaughter; dependent variables: occurrence of ExPEC, antimicrobial resistance, pathotyping, phylogenetic grouping, integrons and virulent genes in ExPEC strains). Correspondence analyses (Greenacre, 2007) were performed using XLSTAT software (version 2023.1.1.1404, Addinsoft, New York, USA) (Seakamela et al., 2022). Contingency tables, and p-values were included as part of test of Independence results.

CHAPTER 4: RESULTS

4.1 Overall prevalence of *Escherichia coli*

A total of 404 chicken carcass samples were microbiologically tested for the presence of *E. coli*. Of these, 93% (n= 376) tested positive for *E. coli* (Table 9) with the highest contamination level at MPN values of >3.04 log cfu/g and the lowest level at MPN value of <-0.48 log cfu/g. Stratification by slaughter type revealed that 52% (n= 194) of the *E. coli*-positive samples originated from chickens slaughtered under informal conditions, where, the contamination level was MPN value of 2.89 log cfu/g , while 48% (n= 182) were associated with formal abattoir processing with the contamination level at MPN value of 2.62 log cfu/g. However, the observed difference in contamination rates between informal and formal abattoir-slaughtered carcasses was not statistically significant (p= 0.1792) (Table 9).

Geospatial analysis of the prevalence revealed notably high contamination rates across all sampled districts. Specifically, 100% of chicken carcasses sampled from East Rand (n= 80), Tshwane (n= 80), and West Rand (n= 84) tested positive for *E. coli*, whereas a slightly lower, yet still substantial, contamination rate was observed in Randfontein 95% (n= 76/80). These regional differences in prevalence were statistically significant (p< 0.0001) with contamination level ranging from MPN values of 2.0 log cfu/g to >3.04 log cfu/g (Table 9).

Table 9: Comparative analysis of *Escherichia coli* prevalence in formal and informal abattoirs across various regions of Gauteng province (n= 404)

Origin of samples	Designation	No. of positive *E. coli* (%)	Average log number	P-value
Slaughter designation	Informal (n=198)	194 (98%)	2.89cfu/g	0.1792
	Formal (n=206)	182 (88%)	2.62cfu/g	
	Total (n=404)	376 (93%)	2.76cfu/g	
Region (Location)	East Rand (n=80)	80 (100%)	>3.04cfu/g	<0.0001
	Ekurhuleni (n=80)	56 (70%)	2.0cfu/g	
	Randfontein (n=80)	76 (95%)	2.66cfu/g	
	Tshwane (n=80)	80 (100%)	>3.04cfu/g	
	West Rand (n=84)	84 (100%)	>3.04cfu/g	

4.2 The presence of Intestinal and Extraintestinal pathogenic *Escherichia coli* in chicken carcasses and animal farm isolates

A comprehensive analysis of the isolates obtained from chicken meat carcasses revealed that the majority, 96% (n= 360/376), were classified within the IPEC pathogroup, whereas a smaller proportion, 4% (n= 16/376), were identified as ExPEC strains (Figure 11). Stratification by slaughter source demonstrated that ExPEC strains were predominantly isolated from formal abattoir-slaughtered chickens, comprising 63% (n= 10/16) of the total ExPEC isolates, while 38% (n= 6/16) originated from informal abattoir-slaughtered chickens. In contrast, IPEC isolates were more frequently recovered from informal-slaughtered carcasses, representing 52% (n= 188/360), compared to 48% (n= 172/360) from formal abattoir-slaughtered carcasses.

Geographical analysis showed that no ExPEC isolates were recovered from chicken carcasses originating from the East Rand or Randfontein regions. However, ExPEC strains were detected in 50% (n= 8/16) of the samples from West Rand, Tshwane 25% (n= 4/16), and Ekurhuleni 25% (n= 4/16) (Figure 11). Although these differences were not significant, a marginal association was observed between geographic distribution and the presence of ExPEC strains (p= 0.0133).

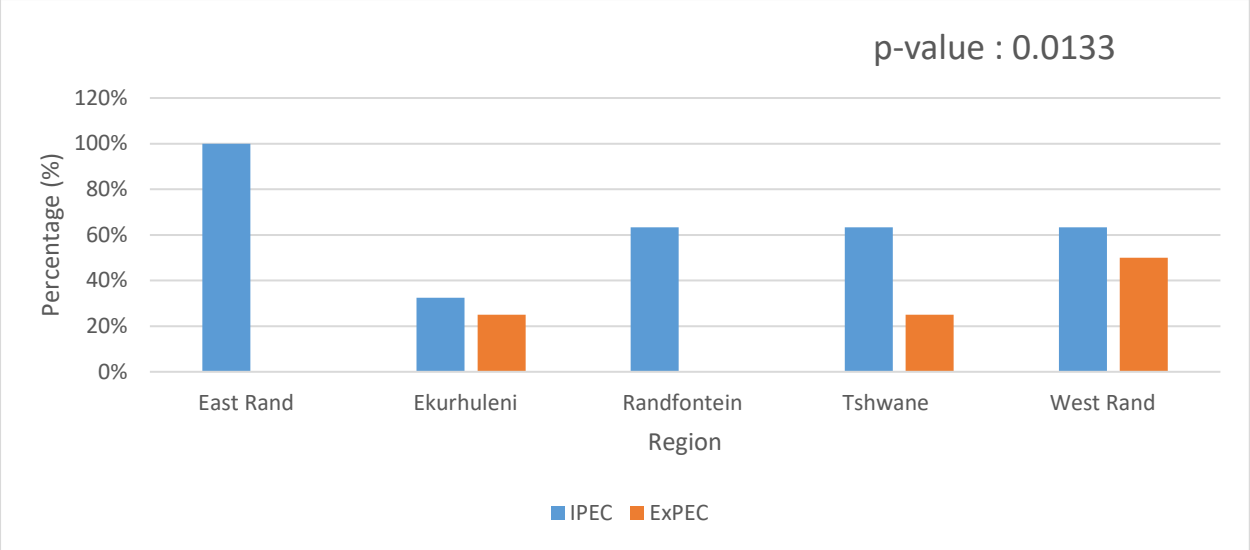

Figure 11: Illustration of IPEC and ExPEC contamination level against geographical origin

To enhance the statistical power and comprehensiveness of the dataset, an additional 63 archived freeze-dried isolates from farm animals were revived and included in the study. Of these, 73% (n= 46/63) were confirmed to be positive for *E. coli*. Further characterization revealed that 85% (n= 39/46) of the revived isolates belonged to the IPEC group, while 15% (n= 7/46) were categorized as ExPEC strains (Figure 12). Overall, the cumulative analysis of all isolates (n= 467) from both chicken meat carcasses and revived freeze-dried samples demonstrated that 90% (n= 422/467) were members of the IPEC pathogroup, whereas 6% (n= 23/467) were classified as ExPEC strains.

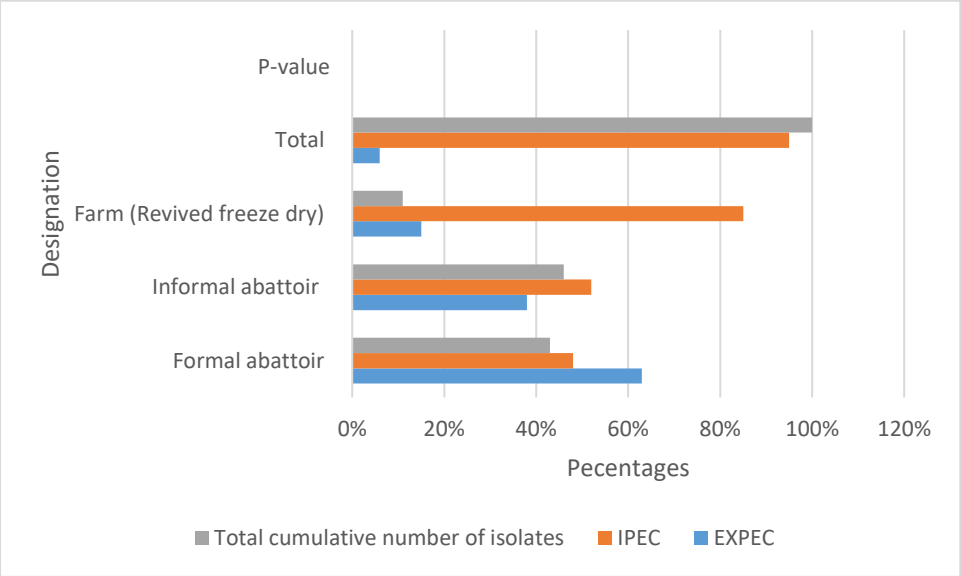

Figure 12: Distribution of IPEC and ExPEC according to the designation of slaughter

4.3 Phenotypic detection of Extraintestinal Pathogenic *Escherichia coli* isolates according to their overall resistance, designation of slaughter and multidrug resistance

4.3.1 Overall phenotypic resistance in Extraintestinal Pathogenic *Escherichia coli* isolates

Antimicrobial susceptibility testing of 23 ExPEC isolates against a panel of 12 antimicrobial agents is summarized in figure 13. The highest antibiotic resistance was observed against tetracycline, with 57% (n= 13/23) of isolates exhibiting phenotypic resistance. This was followed by resistance to ciprofloxacin at 43% (n= 10/23), and both gentamicin and amoxicillin/clavulanic acid each at 39% (n= 9/23) each. In contrast, lower resistance rates were recorded for third-generation cephalosporins such as ceftriaxone, ceftiofur, and cefoxitin 9% (n= 2/23) each, while azithromycin exhibited the lowest resistance at 4% (n= 1/23). Notably, no resistance was observed to sulfisoxazole and trimethoprim/sulfamethoxazole among the ExPEC isolates. The antimicrobial resistance profiles of ExPEC isolates varied significantly across the antibiotics tested ($p < 0.0001$).

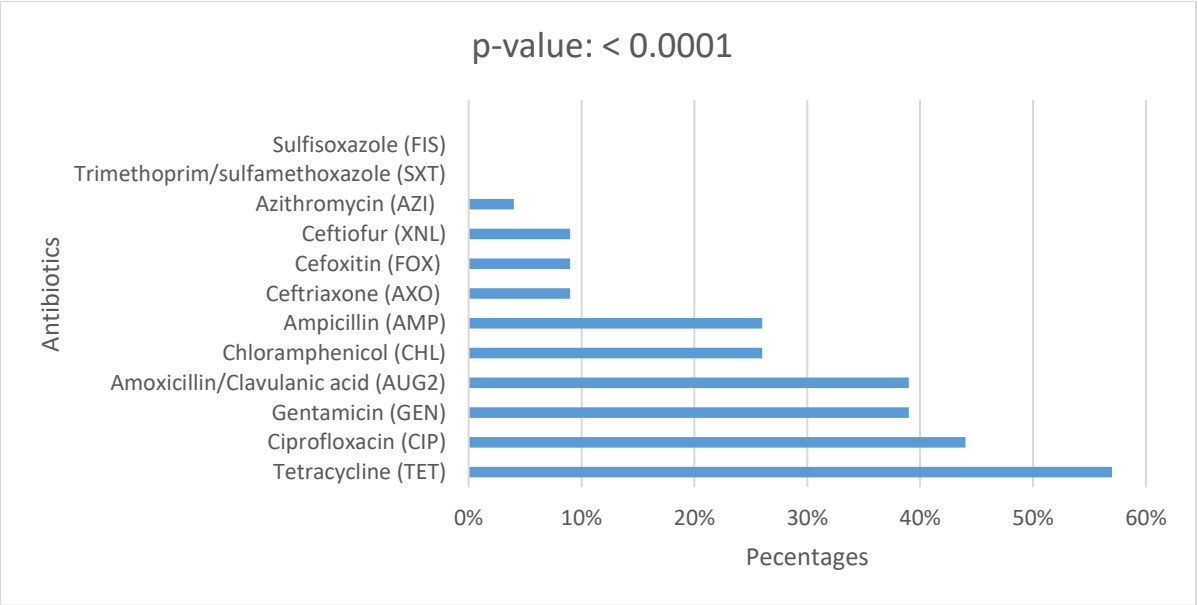

Figure 13: Phenotypic resistance profile of ExPEC isolates against a panel of 12 antimicrobial agents

4.3.2 Phenotypic resistant of Extraintestinal pathogenic *Escherichia coli* according to designation of slaughter

The isolates from formal abattoirs 58% (n= 35/60) demonstrated the highest antimicrobial resistance among various slaughter designations, followed by informal abattoirs 33% (n= 20/60) and lastly freeze-dried 8% (n= 5/60). Moreover, it was found that 14% (n= 7/35) of the isolates from formal abattoirs were resistant to tetracycline followed by ciprofloxacin and amoxicillin/clavulanic acid 17% (n= 6/35), ampicillin and gentamicin 14% (n= 5/35). Among informal designation of slaughter, ciprofloxacin was the highest with 20% (n= 4/20) (Table 12). Tetracycline was also the highest found in freeze dried isolates. The distribution of antibiotic resistance in ExPEC isolates was significant as it varied according to their designation of slaughter ($p < 0.0001$) (Table 10).

Table 10: The relationship between antibiotics and the source of isolates

Antibiotic	Source of isolates (n=23)			P-value
	Formal Abattoir (n=10)	Informal Abattoir (n=6)	Farm Freeze dry (n=7)	
Ampicillin	5 (50%)	1 (17%)	0 (0%)	<0.0001
Amoxicillin/Clavulanic acid	6 (60%)	3 (50%)	0 (0%)	
Ceftriaxone	1 (10%)	1 (17%)	0 (0%)	
Azithromycin	0 (0%)	0 (0%)	1 (14%)	
Chloramphenicol	3 (30%)	2 (33%)	1 (14%)	
Ciprofloxacin	6 (60%)	4 (67%)	0 (0%)	
Cefoxitin	1 (10%)	1 (17%)	0 (0%)	
Gentamicin	5 (50%)	4 (67%)	0 (0%)	
Tetracycline	7 (70%)	3 (50%)	3 (43%)	
Ceftiofur	1 (10%)	1 (17%)	0 (0%)	
Total cumulative resistant isolates per designation	35 (58%)	20 (33%)	5 (8%)	60

4.3.3 Phenotypic resistance patterns and multi-drug resistance profile

Among the 23 ExPEC isolates analysed in this study, a total of 13 distinct AMR profiles were identified. Notably, resistance to three or more antimicrobial agents — indicative of multidrug resistance (MDR) with classes including tetracycline, fluoroquinolones, aminoglycoside, β-lactams and cephalosporin— was observed across all designations. Resistance to five antibiotics was the most common resistance phenotype observed in four individual isolates (Table 11). Among these, two originated from West Rand and Ekurhuleni formal abattoirs falling under five antimicrobial classes (tetracycline, fluoroquinolones, aminoglycoside, phenicol's and β-lactams), while the other two isolates originated from Tshwane and Ekurhuleni informal abattoirs falling under five antimicrobial classes (tetracycline, fluoroquinolones, aminoglycoside, phenicol's and cephalosporin) which is indicative of extensively drug-resistance (XDR). One abattoir-derived isolate from Tshwane municipality exhibited pan drug-resistance (PDR) across all tested antimicrobial classes representing seven antibiotics, specifically TET, CIP, CHL, AXO, AUG, XNL, and AMP falling six under (tetracycline, fluoroquinolones, phenicols, cephalosporin, aminoglycoside and β-lactams). This was followed by an isolate from formal abattoir in the West Rand municipality showing resistant to six antimicrobials (TET, CIP, CHL, AUG, GEN, AMP) falling under (tetracycline, fluoroquinolones, phenicol's, aminoglycoside and β-lactams). Another exhibited resistance to four agents (TET, FOX, AUG, GEN), from formal abattoir in Tshwane municipality falling under three antimicrobial classes (tetracycline, cephalosporin, aminoglycoside).

Additionally, six individual isolates demonstrated resistance to three antimicrobial agents, representing the lowest threshold for MDR categorization. Of these lowest threshold isolates, two were isolated from informal abattoir in West Rand and one in Ekurhuleni, while one was isolated from formal abattoirs in West Rand, Ekurhuleni and freeze-dry. The distribution of MDR ExPEC isolates by designation revealed a higher prevalence in formal abattoir samples 54% (n= 7/13), followed by informal abattoir 39% (n= 5/13) and freeze dried slaughtered 8% (n= 1/13). Majority of MDR isolates from various regions were found to originate from the municipality in West Rand (n= 5/5), followed by Ekurhuleni (n= 4/5).

Table 11: Antibiotic resistance patterns of Extraintestinal pathogenic *Escherichia coli* from various designation and location

Resistant patterns	Number of antibiotics agents in pattern	Number of isolates	Designation	Region/ Location
TET, CIP, CHL, AXO, AUG, XNL, AMP	7	1	Formal Abattoir	Tshwane
TET, CIP, CHL, AUG, GEN, AMP	6	1	Formal Abattoir	West Rand
TET, CIP, CHL, AUG, AMP	5	1	Formal Abattoir	West Rand
TET, CIP, CHL, AXO, GEN	5	1	Informal Abattoir	Tshwane
TET, CIP, FOX, AUG, GEN	5	1	Informal Abattoir	Ekurhuleni
TET, CIP, AUG, GEN, AMP	5	1	Formal Abattoir	Ekurhuleni
TET, FOX, AUG, GEN	4	1	Formal Abattoir	Tshwane
CIP, GEN, XNL	3	1	Informal Abattoir	West Rand
CHL, AUG, GEN	3	1	Informal Abattoir	West Rand
TET, CIP, AUG	3	1	Formal Abattoir	West Rand
TET, AUG, AMP	3	1	Informal Abattoir	Ekurhuleni
TET, CIP, AMP	3	1	Formal Abattoir	Ekurhuleni
TET, CHL, AZI	3	1	Freeze dried	Freeze dried

TET: Tetracycline, CIP: Ciprofloxacin, CHL: Chloramphenicol, AUG: Amoxicillin/Clavulanic acid, GEN: Gentamicin, XNL: Ceftiofur, AMP: Ampicillin, AXO: Ceftriaxone, FOX: Cefoxitin, AZI: Azithromycin

4.4 Detection of resistance genes in Extraintestinal pathogenic *Escherichia coli* from various designation

A total of 23 ExPEC isolates were screened for the presence of 18 antibiotics resistance genes using PCR. The sulfonamide resistance gene *sul3* was the most frequently detected, present in 44% (n= 10/23) of isolates, followed by *sul2* 17% (n= 4/23). In contrast, *sul1* was detected in only one isolate 4%. Among the tetracycline resistance genes, *tetA* was identified in 39% (n= 9/23) and *tetB* in 17% (n= 4/23) of isolates. Of the quinolone resistance genes (*qnrA, qnrB, qnrS*), only *qnrS* was detected, found in 17% (n= 4/23) of isolates. The trimethoprim resistance gene *dfrA2* was identified in 9% (n= 2/23) of isolates, whereas *dfrA1* and *dfrA3* were not detected. Resistance genes encoding phenicols were the least prevalent, with only *cmlA* detected in one isolate (4%), and no detection of *cat1* or *floR*. None of the β-lactamase genes (*blaCMY, blaTEM, blaSHV, blaPSE*) were found in any of the isolates 0%.

When stratified by designation, isolates from formal abattoirs harboured the highest number and diversity of resistance genes. These included *tetA* 17% (n= 4/23), *tetB* 13% (n= 3/23), *sul3* 17% (n= 4/23), *sul1* 4% (n= 1/23), *qnrS* 13% (n= 3/23), and *cmlA* 4% (n= 1/23). Isolates from animals slaughtered at informal abattoir showed a similar pattern, with *tetA* 22% (n= 5/23), *sul3* 26% (n= 6/23), *qnrS* 4% (n= 1/23), *cmlA* 4% (n= 1/23), and *dfrA2* 9% (n= 2/23). Freeze-dried isolates exhibited the lowest prevalence and diversity, with only *tetB* 4% (n= 1/23), *sul1* 4% (n= 1/23), and *sul2* 13% (n= 3/23) detected. Although the distribution of resistance genes appeared to vary across sources, statistical analysis revealed no significant association between designation and the presence of resistance genes (p= 0.0527) (Figure 14).

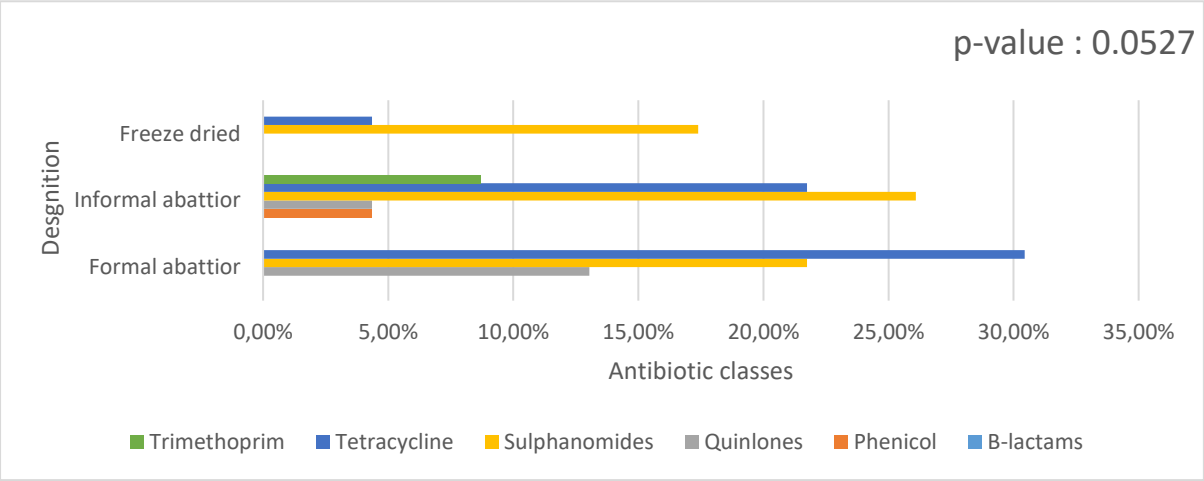

Figure 14: Association between resistance gene detection and the designation

4.5 Overall presence of virulent genes in Extraintestinal pathogenic *Escherichia coli* isolates

The presence and distribution of virulence genes were investigated in 23 bacterial isolates using PCR targeting 18 specific virulence genes. The most prevalent virulence marker was *ompA* gene, which was detected in all isolates 100% (n= 23/23). This was followed by *mat* 83% (n= 19/23), *kpsM* 65% (n= 15/23), *csg* and *sitA* 57% (n= 13/23), and *sat* and *traT* 52% (n= 12/23). All other virulence genes were detected at frequencies below 50%, while *cnf1/cnf2*, *iroN*, and *iucD* were not detected in any of the isolates (Table 12). The overall distribution of virulence genes among isolates was statistically significant ($p < 0.0001$).

When stratified by the designation, most isolates from formal abattoirs, had the highest frequency of *ompA*. This was in contrast to 48 isolates from the informal abattoir and those from the farm (freeze-dried). It was observed that the difference was not statistically significant ($p= 0.0397$) between these locations/regions. Therefore, further analysis of these isolates from these locations was not done due to lack of metadata of freeze-dried samples.

Table 12: Distribution of virulence genes among Extraintestinal pathogenic *Escherichia coli* isolates from various designation.

Antibiotic	Source of isolates (n=23)			Total	P-value
	Formal abattoir	Informal abattoir	Freeze dry		
afa/dra	5 (22%)	1 (4%)	0 (0%)	6 (26%)	< 0.0001
csgA	7 (30%)	5 (22%)	1 (4%)	13 (57%)	
cvi/cva	1 (4%)	1 (4%)	0 (0%)	2 (9%)	
fimH	1 (4%)	0 (0%)	0 (0%)	1 (4%)	
fyuA	1 (4%)	1 (4%)	4 (17%)	6 (26%)	
hlyA	2 (9%)	2 (9%)	0 (0%)	4 (17%)	
ibeA	1 (4%)	0 (0%)	0 (0%)	1 (4%)	
iha	1 (4%)	0 (0%)	1 (4%)	2 (9%)	
irp2	4 (17%)	0 (0%)	1 (4%)	5 (22%)	
iss	0 (0%)	0 (0%)	3 (13%)	3 (13%)	
iutA	5 (22%)	0 (0%)	2 (9%)	7 (30%)	
kpsm	3 (13%)	6 (26%)	6 (26%)	15 (65%)	
mat	8 (35%)	5 (22%)	6 (26%)	19 (83%)	
ompA	10 (44%)	6 (26%)	7 (30%)	23 (100%)	
papA	3 (13%)	2 (9%)	0 (0%)	5 (22%)	
papC	1 (4%)	1 (4%)	6 (26%)	8 (35%)	
Pic	3 (13%)	3 (13%)	0 (0%)	6 (26%)	
sat	3 (13%)	5 (22%)	4 (17%)	12 (52%)	
sfa/foc	2 (9%)	0 (0%)	0 (0%)	2 (9%)	
sitA	8 (35%)	5 (22%)	0 (0%)	13 (57%)	
traT	5 (22%)	3 (13%)	4 (17%)	12 (52%)	
vat	6 (26%)	2 (9%)	3 (13%)	11 (43%)	
Total	80	48	48	176	0.0397

4.6 Distribution of phylogenetic groups isolated in Extraintestinal pathogenic *Escherichia coli* isolates

The phylogenetic classification of ExPEC isolates revealed that majority of them belonged to group A accounting for 39% (n= 9/23) of the isolates, followed by group B2 at 35% (n= 8/23), and groups B1 and D, each representing 13% (n= 3/23) of the isolates (Table 13). When stratified by designation, the distribution of phylogenetic groups among positive isolates was as follows: formal abattoir at 44% (n= 10/23), farm at 30% (n= 7/23), and informal at 26% (n= 6/23). This distribution was statistically significant, with a p-value of <0.0001 which indicates a strong association between slaughter source and phylogenetic grouping.

Table 13: Distribution of phylogenetic groups of Extraintestinal pathogenic *Escherichia coli* isolates

Designation	**Phylogenetic group (%)**				**Total**
	Group A	**Group B1**	**Group B2**	**Group D**	
Formal abattoir	4 (40%)	1 (10%)	2 (20%)	3 (30%)	10 (44%)
Informal abattoir	4 (67%)	2 (33%)	0 (0%)	0 (0%)	6 (26%)
Farm	1 (14%)	0 (0%)	6 (86%)	0 (0%)	7 (30%)
Total	9 (39%)	3 (13%)	8 (35%)	(13%) 3	23

4.7 Occurrence of integrons in Extraintestinal pathogenic *Escherichia coli*

Extraintestinal pathogenic *Escherichia coli* isolates were screened for the presence of class 1, class 2, and class 3 integrons. Class 3 integrons were found in 74% (n = 17) of isolates, identified by the presence of the *intI3* gene. Class 1 integrons (*intI1*) were found in 35% (n = 8) of isolates, while class 2 integrons (*intI2*) were detected in only 9% (n = 2). The presence of integrons was statistically significant with the source of the isolate ($p < 0.0001$). Co-occurrence of class 1 and class 3 integrons was observed in 17% (n = 4) of ExPEC. A single isolate harboured all three classes of integrons (Figure 15).

When analysed by designation, the formal abattoir-derived isolates harboured the majority of integrons. Specifically, in formal abattoir-sourced isolates where class 3 integrons was present in 47% (n = 8), class 1 in 67% (n = 4) and class 2 in 100% (n = 2) of the isolates. In informal

abattoir sourced isolates, class 3 and class 1 integrons were each detected in 29% (n = 5) and 33% (n = 4) of samples respectively. Only class 3 integrons 17% (n = 4) were detected in freeze dry isolates (Figure 15). Despite these observed differences, the association between integron class distribution and designation was not statistically significant (p = 0.0424).

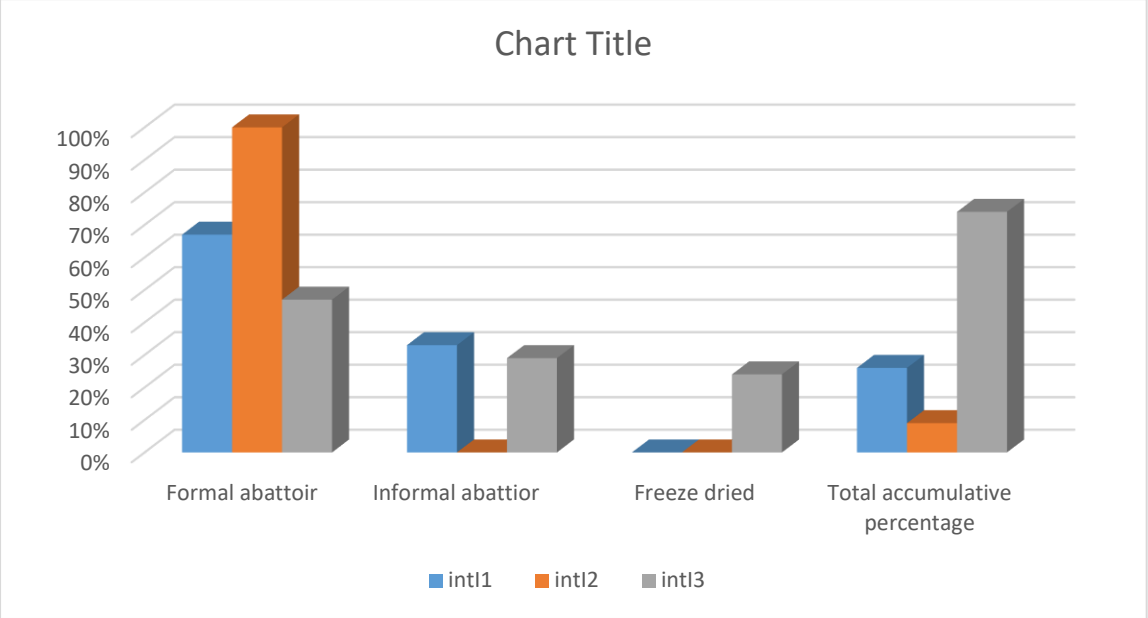

Figure 15 : Percentages of Extraintestinal pathogenic Escherichia coli positive isolates for class 1, 2 and 3 integrons (*intI1, intI2, intI3*) against their designation

4.8 Distribution of pathotyping Extraintestinal pathogenic *Escherichia coli*

According to pathotyping of isolates in this study, it was determined that 91% of the isolates harboured virulence genes that are characteristic of defined ExPEC pathotypes (Figure 16). Based on the virulence-associated genes distribution of pathotypes detected among these isolates, there was equal distribution for Avian Pathogenic *E. coli* (APEC), Uropathogenic *E. coli* (UPEC), and Septicaemic *E. coli* (SEPEC) at 29% (n= 6/21) each, while Neonatal Meningitis-associated *E. coli* (NMEC) was 14% (n = 3/21). The majority of these pathotypes were identified in isolates obtained from formal abattoir-slaughtered animals 48% (n = 10/21), followed by those from farms (33%, n = 7/21), and lastly, informal abattoir slaughter 19% (n = 4/21). However, statistical analysis showed no significant association between pathotype distribution and designation (p= 0.6445).

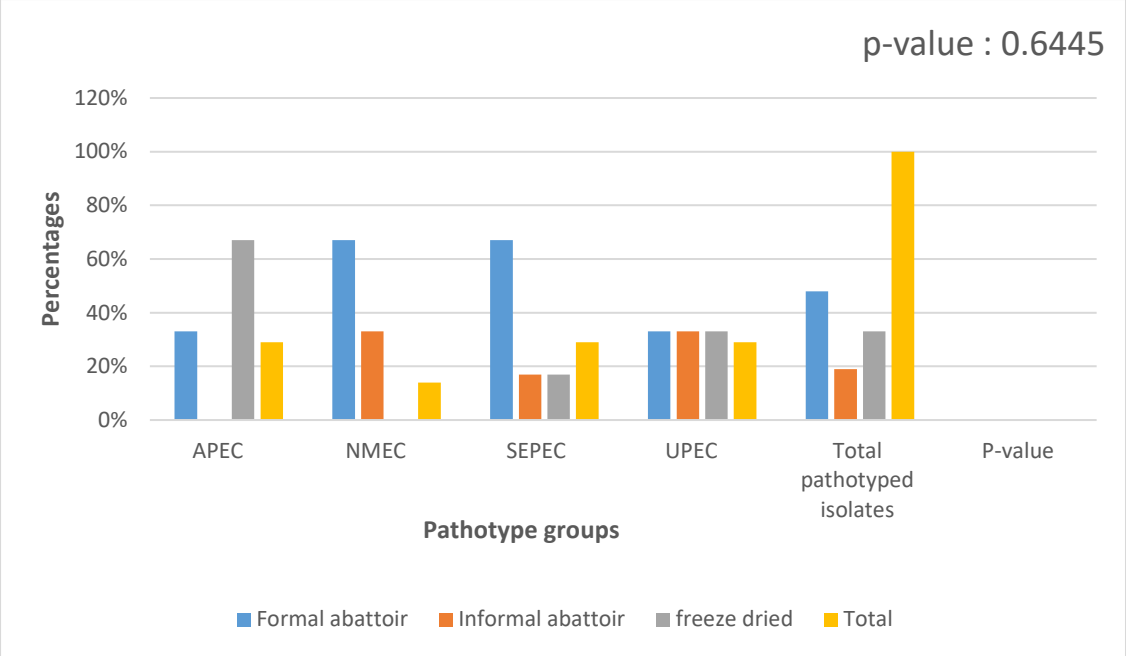

Figure 16: Occurrence of Extraintestinal Pathogenic *Escherichia coli* pathotypes

4.9 Cluster analysis of Extraintestinal pathogenic *Escherichia coli* isolate

The asymmetric row plot of correspondence analysis (CA) plot shows the total of 100% percent variance explained by the first two dimensions, (F1 and F2) depicted in Figure 17 and 18 indicating data representation. Figure 17 illustrates the relationship between ExPEC pathotypes, phylogroups and integrons, indicating that GrpA correlates significantly with informal designations, while GrpB1 is associated exclusively with informal abattoir designations. SEPEC, *intI1*, and NMEC exhibit a stronger link to formal abattoir designations. The bottom of the F2 dimension shows that *intI2* and GrpD are exclusively related to formal abattoir designations, while *IntI3* is common across all slaughter designations. UPEC connects to both informal and freeze-dry designations, whereas GrpB2 and APEC are only associated with freeze-dry designations on the right side of the F1 dimension.

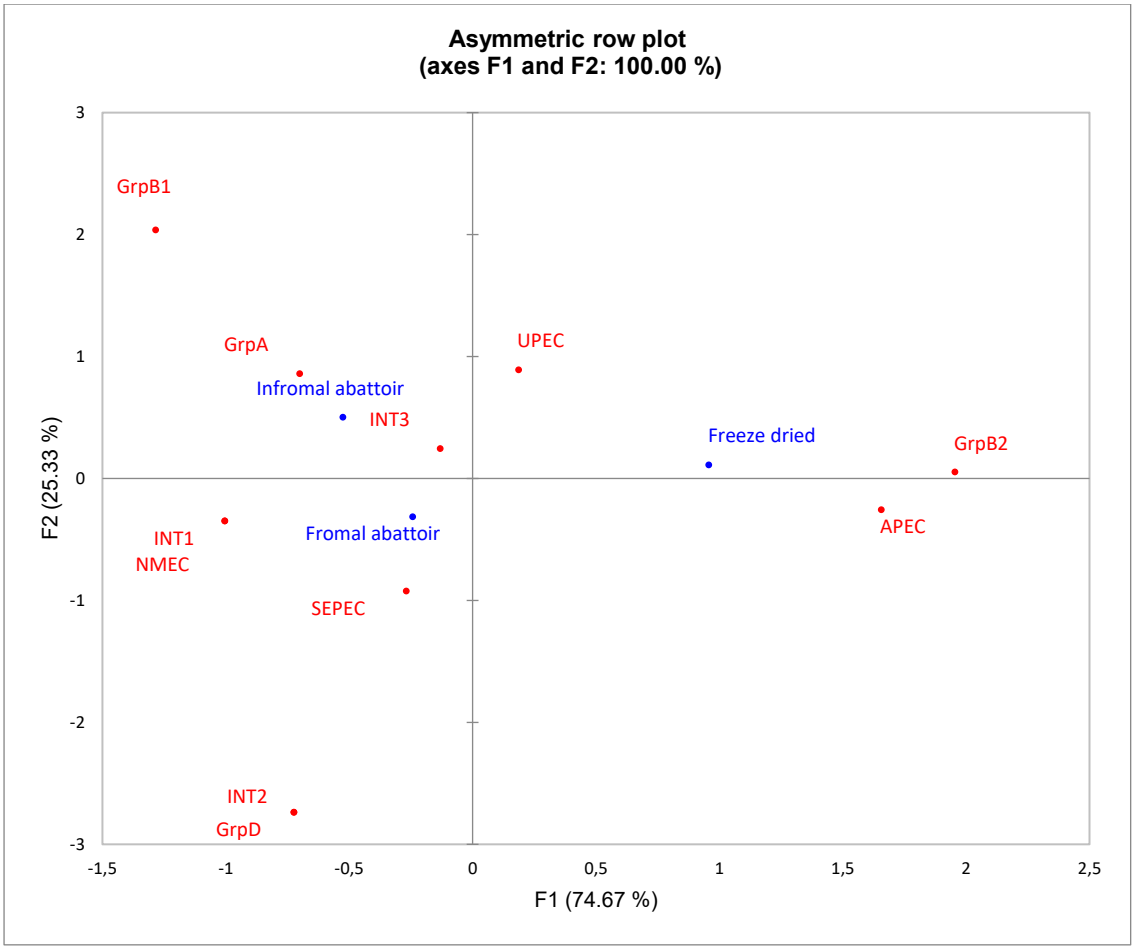

Figure 17: Asymmetric plot of integrons, phylogenetic groups and pathotyping

In figure 18, analysis of resistance genes indicates that *cm1A* is exclusively associated to formal abattoir slaughter, while *qnrs* is significantly associated with formal abattoirs over informal. Conversely, *tetA* and sul3 are more prevalent in informal abattoirs, and *Drf* is found only in informal settings. On the F1 dimension, *tetB* has a stronger association with formal abattoirs compared to freeze dry, whereas *sul1* and *sul2* are mainly linked to freeze dry. Additionally, the right side of the F1 dimension shows that *tetB* is more closely associated with abattoirs than farms, while sul1 and sul2 are primarily associated with the freeze dry designation of slaughter.

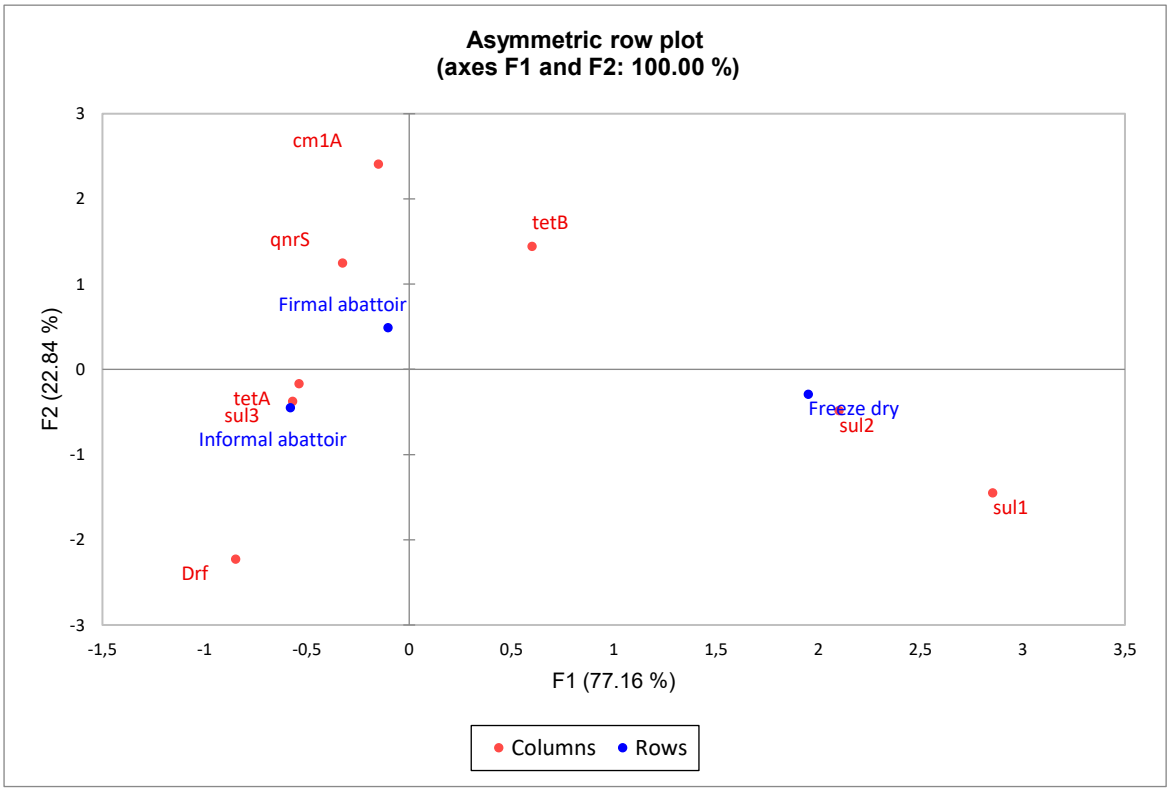

Figure 18: Asymmetric plot of resistant genes in various designation of slaughter

Analysis of virulent genes depicts distinct associations with various designations across dimensions of a plot in figure 19. The left side of F1 connects *pic*, *hlyA*, and *cvi*/*cva* genes to informal abattoir designations, while *papA*, *csgA*, and *sitA* are more aligned with informal than formal abattoirs. The bottom side of F2 links *ibeA*, *fimH*, and *sfa*/*foc* solely to formal abattoirs, with *afa*/*dra* more strongly tied to formal than informal abattoirs. Genes *irp2* and *iutA* are associated more with formal abattoirs than freeze dry, while *iha* is linked to freeze dry. Common genes across all designations, located near the plot's origin, include *OmpA*, *vat*, *mat*, and *traT*. Additionally, the top side of F2 shows sat and *kpsM* associated more with informal than freeze dry, and the right side of F1 indicates *fyuA*, *papC*, and *iss* are linked to freeze dry, while *tetB* is more associated with formal abattoirs and *sul1* and *sul2* with freeze dry.

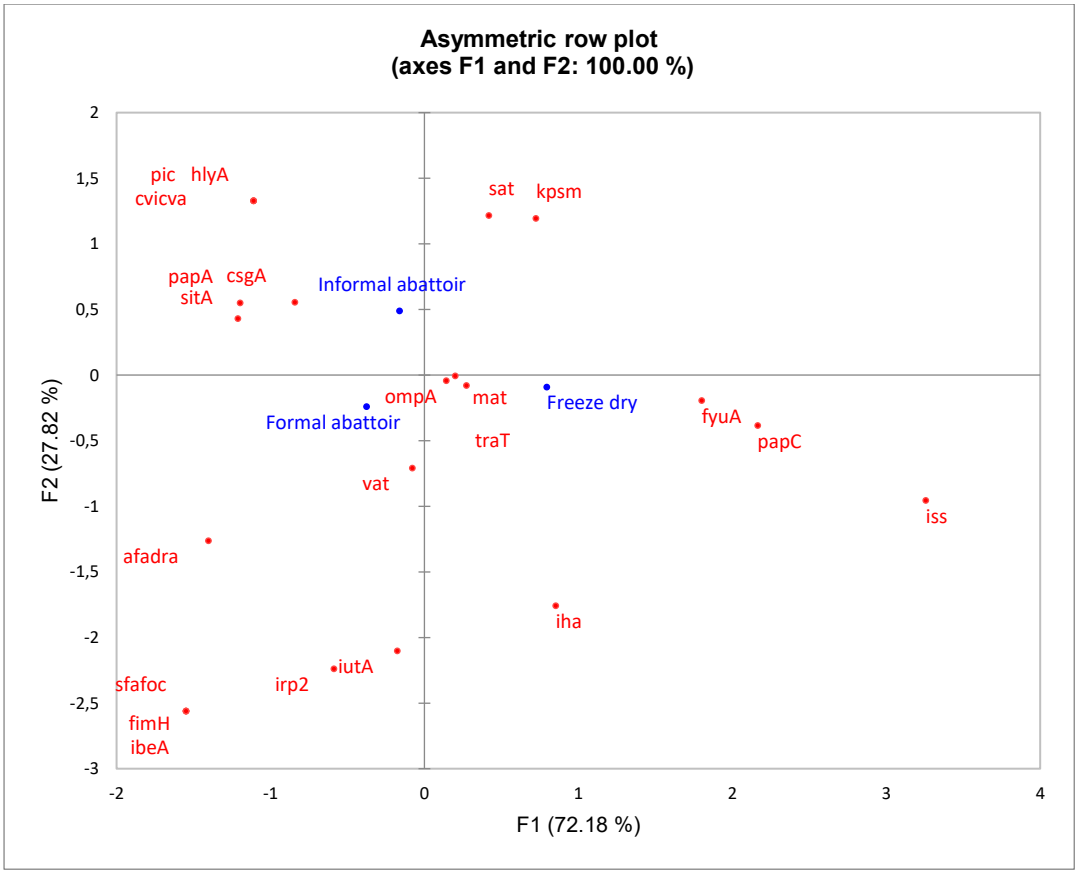

Figure 19: Asymmetric distribution of virulent genes in various designation of slaughter

CHAPTER 5: DISCUSSION

5.1 Overall prevalence and contamination levels of *Escherichia coli*

This study investigated the prevalence of *E. coli* in chicken carcasses from Gauteng Province. The overall prevalence of *E. coli* was 93%, which was extremely high. This high prevalence is concerning primarily because the presence of *E. coli* in abattoir carcasses serves as an indicator of fecal contamination during processing, especially during evisceration and reflects deficiencies in abattoir sanitation practices (Liur & Veerman, 2021). Other key factors contributing to the high prevalence of *E. coli* include cross-contamination in abattoirs occurring due to high-speed processing using equipment's (e.g knives and tables) and improper washing (Thomas et al., 2025; Yulistiani et al., 2019). Improper water sanitation practices, such as reuse of cleaning water and contaminated water used for processing or cleaning, exacerbate bacterial accumulation (Ovuru et al., 2024). High stocking densities in farms stress birds, weakening their immune systems and increasing *E. coli* shedding (Risalvato et al., 2025). Persistent environmental reservoirs of *E. coli* in abattoirs survive cleaning efforts, leading to re-contamination (Ovuru et al., 2024). Finally, high throughput in abattoirs reduces cleaning time per carcass, further increasing contamination risk. Beyond its role as a hygiene indicator, *E. coli* holds considerable a public health importance due to pathogenicity of other strains, which are implicated in foodborne illnesses (Lupindu, 2017; Morshdy et al., 2024).

The findings of this study are similar to the one reported by Machedi (2020) where 94% of boiler meat in Durban abattoir was contaminated with *E. coli*. Similarly, Kagambega et al. (2011) reported a prevalence of 75% in broiler meat and meat products in Pretoria, Gauteng. The presence of *E. coli* in both studies by Machedi et al. (2020) and Kagambega et al. (2011) was linked to a lack of hygiene and sanitation during slaughter processes, which could also be the case in this study. This is further supported by a study conducted by McIver et al. (2020), which found that the high prevalence of *E. coli* in chicken meat may be attributed to cross-contamination, as well as inadequate hygiene and sanitation during slaughter processes.

The enumeration of *E. coli* in meat and meat products is widely recognized as a reliable measure of hygiene standards during meat processing (Enver et al., 2021). Therefore, the contamination level of *E. coli* in tested samples which ranged from MPN values of 2.0 log

cfu/g to >3.04 log cfu/g, indicated an alarming level of microbial load. According to the Veterinary Procedural Notice 56 standard, ideally *E. coli* should be detected in a level of <3cfu/g (the limit of the Most Probable Number test = 0.5log cfu/g) which has been provided to be a satisfactory criterion for *E. coli.* Levels exceeding ≥100cfu per Gram (≥2.0 log cfu/g) are unacceptable, which indicates poor hygiene or poor food handling practices, potential contamination, as a result introducing pathogens or allowing them to survive in food before processing "Guidelines for the Microbiological Examination of Ready-to-Eat Foods" (2001). The presence of unacceptable levels of *E. coli* in this study poses serious public health risks. The implications of *E. coli* consumption of contaminated chicken meat can results in foodborne illnesses (UTIs: cystitis pyelonephritis; bloodstream infections: sepsis; meningitis; pneumonia) in humans, including symptoms such as burning pee and frequent urination (UTI), fever, chills, low blood pressure (sepsis), headache, stiff neck and fever (meningitis) diarrhea, vomiting, and abdominal pain (Kathayat et al., 2021). Furthermore, populations such as young children, the elderly and immunocompromised individuals which represent relatively high numbers in Gauteng may be prone to severe disease (Percival & Williams, 2014).

The study revealed a prevalence of 52% in samples from informal abattoirs and 48% in those from formal abattoirs, indicating no statistically significant difference. A finding of no statistical difference between 52% contamination in informal abattoirs and 48% in formal abattoirs indicates that the 4% difference is too small to be considered a true reliable difference (Kafi & Ansari-Lari, 2022). Contributing factors to *E. coli* contamination in abattoirs include poor hygiene adherence, contaminated water, insufficient staff sterilization training, inadequate maintenance of equipment, and cross-contamination (Yulistiani et al., 2019; Thomas et al., 2025). The implications of this are the public health risk with the contamination levels of 52% versus 48% suggesting both abattoirs pose comparable risks of transmitting *E. coli* through food chain (Sebsibe & Asfaw, 2020).

The prevalence of *E. coli* varied across municipalities, generally exceeding 70%, which indicates widespread poor hygiene practices across the province largely influenced by the operations of informal abattoirs. The deterioration of wastewater treatment and sewage pipe networks is the primary reason for elevated *E. coli* in Gauteng (Makuwa, 2022). Aging infrastructure causes bursts and leaks, leading to groundwater and surface water contamination. Informal settlements exacerbate the issue with inadequate sewage disposal, resulting in open defecation and waste dumping near water bodies. Rainfall increases the spread of contaminants

into water sources, heightening *E. coli* levels, especially during the rainy season. Additionally, high regional temperatures facilitate *E. coli* survival and reproduction, while feces from free-ranging animals in peri-urban areas further contribute to contamination near agricultural zones (Kim et al., 2025). This underscores the urgent need to review and strengthen hygiene management systems within these facilities. Paradoxically, Gauteng Province, despite its reputation for industrial development and advanced urban infrastructure, also hosts a substantial number of under-resourced informal abattoirs. These facilities play a vital role in local economies and cultural traditions, particularly during high-demand periods such as traditional ceremonies, weddings, and funerals, when the demand for fresh meat increases sharply (Nkosi, 2020). However, many informal abattoirs operate on the margins of regulatory compliance, struggling to meet the standards set by the Meat Safety Act due to persistent infrastructure deficits, inadequate access to veterinary services, and limited government support. In practice, these facilities often lack essential equipment, trained personnel, effective cold chain systems, and hygienic environments necessary for safe meat processing, thereby increasing the risk of foodborne pathogen transmission and compromising public health.

5.2 The presence of Intestinal and Extraintestinal pathogenic *Escherichia coli* in chicken carcasses and freeze-dried isolates

Based on the molecular characteristic genes (*papA, iutA, foc/sfa, afa/dra, kpsM II* and *papC*), ExPEC was detected in 4% of the chicken meat carcasses while IPEC accounted for 96% of samples. It is no surprise that IPEC accounted for the highest prevalence as *E. coli* is often found in chicken meat as it can easily be transmitted through fecal-oral route. This is evident because IPEC strains colonize the intestines of chicken making them more susceptible to intestinal infections. A study by Lindstedt et al. (2018) revealed that 94% of chicken isolates were classified as belonging to an IPEC pathovar, additionally carried ExPEC virulent genes although IPEC had the highest prevalence at 69%. the high prevalence of IPEC poses challenges to disease control measures, especially when strains are diverse, and can lead to economic losses for farmers due to mobility and motility. Continuous epidemiology is vital for control measures and for food safety and security to human.

5.2.1 Extraintestinal pathogenic *Escherichia coli* in chicken meat isolated from various designation

The presence of ExPEC colonizing chickens in abattoirs could be significant to both animal and human health. Extraintestinal pathogenic *E. coli* strains were primarily from formal abattoir-slaughtered chickens, accounting for 63% of the total isolates, while IPEC isolates were more frequently found in informal-slaughtered carcasses. These findings are rather different from that reported by Jaja et al. (2018), as he indicated that informal abattoirs had higher *E. coli* results than formal abattoirs, whereas this study data indicated that formal abattoirs had higher *E. coli* results than informal abattoirs, this indicates that 'the informal sector's washing of carcasses doesn't significantly reduce microorganism levels in meat, while formal sector's bacterial counts were fewer but often exceeded the regulated benchmark. The high prevalence of ExPEC in formal abattoirs may be due to potentially contaminated equipment and multiple chickens being processed simultaneously, while the low prevalence of IPEC suggests gut clearing prior to slaughter. This finding suggests that the difference in informal slaughter introduces IPEC contamination while abattoirs might have more control in places to prevent this (Gharaibeh et al., 2024). The higher number of ExPEC in formal abattoir isolates suggests a potential higher capacity for these bacteria to cause diseases in humans or animals. The correlation between formal and informal slaughterhouses needs to be further investigated to understand the significance of its potential to cause mobility and mortality in both human and animals.

Using a geographical analysis, ExPEC isolates were detected in chicken carcasses from West Rand, Tshwane, and Ekurhuleni, but not from East Rand or Randfontein. Thus far there is no specific data on ExPEC prevalence in chickens from these regions. The data variation may be influenced by environmental factors like waste management and water sanitation, as well as farming practices like antibiotic use. More research is required in these areas, although, these findings provide valuable insight into the geographical distribution of ExPEC in chicken carcasses, highlighting the need for region-specific interventions to mitigate the risk of ExPEC contamination.

5.3 Phenotypic detection of Extraintestinal Pathogenic *Escherichia coli* isolates according to their overall resistance, designation of slaughter and multidrug resistance

In this study, ExPEC isolates exhibited the highest (57%) resistance to tetracycline, a widely used antibiotic in SA (Jaja et al., 2020). Similarly, McIver et al., (2020) reported findings showing tetracycline (27%) in poultry isolates in KwaZulu-Natal Province. The Fertilizers, Farm, Feeds, Agricultural and Stock Remedies Act (Act 36, 1947) permits over-the-counter purchase of antibiotics such as tetracycline which is relatively cheap and easily accessible by the South African animal producers particularly in commercial chicken production system for growth promotion and therapeutic purposes (Theobald et al., 2019). In SA, Moyane et al. (2013) and Smith et al. (2019) suggested that tetracycline, penicillin, and sulphonamides were the most often overused antibiotics. Therefore, these might be the reasons to the high resistance of the antibiotic in this study.

Withdrawing antibiotics prior to chicken slaughter is a normal procedure that is acceptable, however, it may be difficult to determine if small-scale rural poultry farmers regularly follow this recommendation (Selaledi et al., 2020). Residues from antibiotics can remain in poultry tissues even after a withdrawal period if not accurately followed (Chen et al., 2025). Residues in chicken tissue can persist despite expected diminishment due to inadequate training and oversight regarding withdrawal periods (Andrew Selaledi et al., 2020). This persistence poses public health risks, including antibiotic resistance, and potential long-term health (allergic reactions) consequences for consumers (Chen et al., 2025). Antimicrobial residues have been found in African countries like Egypt, Ethiopia, Ghana, Kenya, Nigeria, SA, Sudan, and Tanzania (Oladeji et al., 2025). A study in Egypt found 44% of fresh chicken samples contained tetracycline residues, some above the Codex Alimentarius maximum residue limit (Van et al., 2019). This is also evidently identified in the rate of tetracycline isolated in chickens from the abattoir designation of slaughter.

The study revealed that 44% of ExPEC isolates exhibited resistance to ciprofloxacin, which is the second highest antibiotic that exhibited resistance in *E. coli*. The resistance levels in *E. coli* isolates from chicken carcasses and feces in South African poultry abattoirs between 2019 and 2020 were found to be low (19%–2%) (Hassan et al., 2024). Similarly, McIver et al. (2020) reported ciprofloxacin resistance at 4% with less prevalence in KwaZulu-Natal Province, SA.

The resistance against ciprofloxacin varies across different countries, with reported resistance rates ranging from 20-30% (Ali, 2010). However, Das et al. (2023), reported a higher prevalence of 78% ciprofloxacin-resistance *E. coli* in Bangladesh, contrary to this study's prevalence rate. Ciprofloxacin is a highly effective antibiotic for the holistic treatment of urinary tract and respiratory tract infections in human (Ali, 2010). The Food and Drug Administration (FDA) has advised against using ciprofloxacin for uncomplicated UTIs due to its potential adverse reactions (Shariati et al., 2022). Ciprofloxacin resistance in uropathogens has significantly increased over the past decade, with *E. coli* resistance rising from 2 to 16% in a 10-year follow-up (Shariati et al., 2022). The increasing resistance to ciprofloxacin in the poultry sector is primarily due to its excessive and indiscriminate use of quinolones (Das et al., 2023). *Escherichia coli* resistance to quinolone antibiotics may be attributed to chromosomal mutations or plasmid-mediated resistance (Kasanga et al., 2024). The reduction in ciprofloxacin susceptibility is crucial as it can potentially lead to treatment failures and pose a public health risk.

Resistance to aminoglycosides (gentamicin and amoxicillin/clavulanic acid) in this research was fairly higher (39%) considering report rates in SA This statement indicates that South African chicken isolates demonstrate a resistance to aminoglycosides ranging from 0% to 10%, which is relatively low compared to findings from this research (Hassan et al., 2024). The prevalence of gentamicin resistance has been reported to vary in a number of foreign nations, including Bangladesh (8-51%), India (25-77%), Pakistan (34-79%), Thailand (20-43%), and Malaysia (20.2-20.3%) (Das, 2020). Gentamicin resistance may be due to apramycin, a veterinary medicine structurally similar to gentamycin, and apramycin-resistance *E. coli* may also be resistance to gentamicin (Ranasinghe et al., 2022).

The rate of resistance to ampicillin was lower (26%) among the β-lactam class. According to Xia et al. (2011), the ExPEC results showed a contrast that 23% of the samples were ampicillin resistance. This is however inconsistent with the findings of Zou et al. (2021), reporting ExPEC isolates with at 87% resistance to ampicillin. The majority of research indicates that ampicillin resistance rates are typically high in poultry meat ranging from 40-100% (Bhave et al., 2019; McIver et al., 2020; Kasanga et al., 2024). Ampicillin is a semi-synthetic β-lactam antibiotic that is frequently used to treat human and livestock *E. coli* infection, but recently its resistance rate has increased (Li et al., 2019). Human UTIs caused by UPEC are often treated with ampicillin, however, due to its frequent usage, resistance has been observed (Meena et al.,

2023). The fact that penicillin is frequently obtained without a prescription and are frequently used improperly may be a factor in the reported resistance to this antibiotic. The existence of AmpC β-lactamases, which are encoded in the *E. coli* chromosome, may also contribute to the bacteria's resistance to penicillin (Kasanga et al., 2024).

Chloramphenicol is a potent antibiotic used to treat severe, life-threatening bacterial infections in poultry (Landoni & Albarellos, 2015). The usage of chloramphenicol has rapidly declined amongst global countries as it was banned due to its long-term toxicity, which results in aplastic anaemia and a decline in bone marrow function (Ng, 2014). In SA, chloramphenicol is highly regulated due to health side effects (inducing suppression of the bone marrow). The FDA has stated that chloramphenicol is not approved for use on food-producing animals. Chloramphenicol (phenicol) had the same resistance rate (26%) as ampicillin. Literature suggests that ampicillin and chloramphenicol have the ability to co-resist (Ranasinghe et al., 2022) and the research findings are in correlation with this statement. The study's prevalence of resistance antibiotic was slightly higher than those of Sarba et al. (2019), Bhave et al. (2019) and McIver et al. (2020) at 17%; 6% and 11%, respectively. The variation of results may be due to the different method used for the prevalence of antimicrobial phenotypic resistance and the geographical location. Regardless of chloramphenicol being banned, it’s still isolated. The mere existence of chloramphenicol may increase antibiotic resistance, which could reduce the antibiotic's therapeutic efficiency.

All ExPEC isolates were 100% susceptible to the class of sulphonamides antibiotics. Sulphonamides are restricted in poultry due to their limited safety margin. They cause bone marrow suppression, thrombocytopenia, lymphoid and immune depression, and post-mortem changes (Landoni & Albarellos, 2015). Additionally, they can cause prohibitive residues in meat and eggs (Landoni & Albarellos, 2015). The class of sulphonamides has a high susceptibility rate, which is attributed by the decrease in the usage of antibiotics within the sulphonamides class. The high susceptibility of sulphonamides to ExPEC indicates their effectiveness and aids healthcare professionals in selecting the most effective antibiotic.

5.3.1 Phenotypic resistance of Extraintestinal pathogenic *Escherichia coli* according to designation of slaughter

According to this study, the highest levels of antimicrobial resistance were discovered in 58% of isolates from formal abattoir (formal abattoirs have higher AMR due to high throughput and cross-contamination from numerous chickens in shared co-housing/slaughter spaces, alongside significant contributions to residues from the extensive use of antibiotics in large-scale farming for growth promotion or disease prevention) (Ren et al., 2025), 33% of isolates from informal abattoir (informal abattoirs often lack good hygiene practices resulting in microbial contamination that may carry lower antibiotic resistance compared to high quantities in formal abattoirs, and 8% of isolates that were freeze-dried. Amongst the isolates, the highest levels of antimicrobial resistance were observed in tetracycline resistance, amoxicillin/clavulanic acid and ciprofloxacin. Singh et al. (2025) have linked improper antibiotic use in poultry farms to significant antibiotic resistance with similar antimicrobial resistance. The fact that formal abattoirs source their chickens from large-scale farms that usually administer antibiotics for growth promotion and disease control might be the reason for the variation levels of antimicrobial resistance due to their standard practices and antibiotic treatment protocols within the abattoirs. The co-selection of resistance determinants and co-resistance of *E. coli* isolates could be another reason for this variation (Jaja et al., 2020). Although informal abattoirs may employ fewer biosecurity protocols, they may also use fewer antibiotics, which could lead to a decrease in the prevalence of antibiotic resistance.

5.3.2 Phenotypic resistance patterns and multi-resistance

Extraintestinal Pathogenic *Esherichia coli* related poultry diseases cause significant food industry losses, but widespread antimicrobial use by poultry farmers has led to multidrug-resistance strains (Lima-Filho et al., 2013). The study revealed that 57% of ExPEC isolates were resistance to at least three antibiotics, indicating a significant antimicrobial resistance pattern. Jaiswal et al. (2024) supported this study findings with *E. coli* isolates showing maximum resistance against tetracycline, ciprofloxacin, trimethoprim, and erythromycin, amoxicillin-clavulanic acid and ampicillin. Multidrug-resistance ExPEC strains were mostly found in formal abattoirs (54%), with lower prevalence in informal abattoirs and freeze-dried isolates. This may be due to unhygienic slaughter practices leading to *E. coli* contamination, primarily due to excessive antibiotic use and mutations (Rahman et al., 2020). One formal

abattoir derived isolate exhibited PDR, which is interesting considering that in Gram negatives PDR is known to be exceedingly rare in *E. coli* (Karakonstantis et al., 2020; Jawad et al., 2024). Pan drug resistance isolates often exhibit resistance to commonly used antibiotics, making it challenging to treat the infection. This study highlights the importance of understanding MDR and PDR patterns in animal production as multidrug resistance ExPEC in chicken abattoirs can contaminate the food chain posing risk to consumers which may compromise human infections treatment effectiveness, as excessive antibiotic use can potentially lead to resistance. Therefore, regular monitoring and surveillance of MDR in chicken abattoirs can aid to identify potential MDR/PDR issues early.

5.3.3 Detection of genotypic antimicrobial resistance genes in Extraintestinal pathogenic *Escherichia coli* isolates

The distribution of tetracycline resistance genes (*tetA*, *tetB*) in ExPEC isolates was detected by utilizing PCR method. Majority of isolates that were resistance to tetracycline antibiotic contained either *tetA* or *tetB* genes. None of the isolates carried both resistance genes, the absence of isolates carrying both resistance genes indicate a limited resistance profile, implying that targeted treatment could be effective as bacterial resistance evolves. This necessitates combination therapy with multiple antibiotics to prevent the bacteria from maintaining both resistance mechanisms and to avoid the development of multi-drug resistance (Urban-Chmiel et al., 2022; Belay et al., 2024). Out of the ExPEC isolates, 39% carried tetracycline resistance genes. The positive rates of *tetA* and *tetB* were 39% and 17%, respectively. Duan et al. (2020) reported positive rates as *tetA* and *tetB* was found in 42% and 17% of ExPEC isolates, respectively. The phenotype's positive rate for tetracycline resistance was relatively consistent with the genotype's positive rate. Tetracycline resistance genes prevalence in ExPEC, is primarily mediated by *tetA* and *tetB*, which are actively present in their resistance mechanism. Efflux pumps are the primary mechanism of tetracycline resistance in Gram-negative bacteria, especially ExPEC. The *tetA* and *tetB* genes create energy-dependent efflux pumps that remove tetracycline from the bacterial cell, reducing its efficacy by preventing binding to the 30S ribosomal subunit (Yi et al., 2022). This allows bacteria to adapt and develop further resistance to tetracycline which is a broad-spectrum antibiotic used to treat various bacterial infections including UTI in SA (Yi et al., 2022).

The ExPEC isolates showed the highest frequency of sulphonamide resistance genes, with *sul3* (44%) being more frequently detected than *sul2* (17%) and *sul1* (4%). Despite the ExPEC isolates exhibiting sulphonamide-resistance genes, these isolates did not exhibit resistance to the sulphonamide antibiotics. This implies that despite the presence of resistance genes, the ExPEC isolates remained susceptible to sulphonamides antibiotics. This also highlights the risk of resistance spread through horizontal gene transfer (Bhat et al., 2023). These "silent" genes do not manifest as resistance in the bacteria but may mislead laboratory screenings into indicating untreatable infections, whereas phenotypic testing can still reveal the efficacy of sulphonamide antibiotics (Postupolski et al., 2021). This may be due to the resistance genes being expressed at a lower level rendering them ineffective against the antibiotics. The resistance genes might be present but may not confer resistance to the specific sulphonamide antibiotics used in this study (Enne et al., 2006; Jian et al., 2021). This study demonstrated that the presence of genotypic resistance does not always translate to phenotypic resistance, highlighting the significance of both phenotypic and genotypic research.

Amongst the quinolones (*qnrA*, *qnrB*, *qnrS*) resistance-encoding genes, only *qnrS* gene was identified in the ExPEC isolates harbouring resistance (17%). Studies by Mahmud et al. (2018) and Amiri et al. (2017) have also shown that *qnrS* is the most common qnr gene identified in isolates from broiler chickens. In poultry, the class of fluoroquinolone antibiotics are utilized frequently to treat *E. coli*-related infections and common empiric treatment in human UTI when first line antimicrobial treatment fails (Zou et al., 2021). Extraintestinal Pathogenic *E. coli*'s resilient resistance to ciprofloxacin and *qnrS* genes, makes it imperative to continuously monitor phenotypic and genotypic resistance in ExPEC. The presence of *qnrS* resistance gene in *E. coli* isolates from chicken meat highlights the need for effective food safety measures to prevent the spread of resistance ExPEC as quinolone antibiotics are used for public health treatment.

Chloramphenicol resistance can be mediated enzymatically or non-enzymatically through *cmlA* or *flo* genes (Bischoff et al., 2005). The *flo* gene, which shares 57% amino acid identity with *cmlA*, ensures the persistence of the resistance phenotype (George, 2002; Bischoff et al., 2005). In the current study, prevalence of the phenicol (antibiotic class) resistance gene in the isolates were exceptionally low. The phenicol genes were detected in one gene (*cm1A* - 4%) amongst the rest of the genes (*cat1* and *flo* - 0%). Li et al. (2007) reported that the occurrence rate of phenicol *E. coli* resistance genes is typically low with only 10% for both *flo* and *cm1A*

genes. Schwarz et al. (2004) revealed that *flo* and *cmlA* genes were present in 71% and 5% of 48 bovine *E. coli* isolates, respectively (Schwarz et al., 2004). The presence of *cmlA* gene provides resistance to chloramphenicol in chickens as it's carried on plasmids by *E. coli*, particularly because *E. coli* can colonize and persist in poultry (Bischoff et al., 2005). This study demonstrates that the genetic linkage between *cmlA* and resistance genes to chloramphenicol agents illustrates persistence of phenotypic resistance. Even though there's a low prevalence of phenicol in chicken meat, along with the presence of *cmlA* genes, it raises concerns about potential transmission of resistance genes transfer to humans through the food chain since phenicols are administered for severe infections in human and has the potential of multidrug resistance.

None of the isolates showed any resistance to the β-lactamase-encoding genes (*blaCMY*, *blaTEM*, *blaSHV*, and *blaPSE*), yet the isolates were resistant to β-lactam-ampicillin (26%) antibiotic. Extraintestinal pathogenic *E. coli* isolates frequently show resistance to β-lactam antibiotics because they contain β-lactamase enzymes, which degrade the β-lactam ring rendering the antibiotics ineffective. This study has demonstrated that, ExPEC isolates can exhibit resistance to ampicillin without necessarily possessing β-lactam resistance genes. Extraintestinal pathogenic *E. coli* isolates can exhibit ampicillin resistance even without β-lactamase resistance genes due to altered porins, efflux pumps, or penicillin-binding protein mutations, using resistance mechanisms instead of producing bactamse (Nasrollahian et al., 2024).

Extraintestinal pathogenic *Escherichia coli* isolates from formal abattoirs harboured the highest number and diversity of resistance genes compared to other designations (informal abattoirs and freeze-dried isolates). The formal abattoirs may provide a favourable environment conducive to genetically exchange between microorganism facilitating the spread of resistance genes (Ahmad et al., 2023). This study reveals that formal meat sector, despite its widespread use of food safety systems and standardization, often fails to meet the microbial quality standards of the informal sector seeing that the genotypic resistance is more prevalent in formal abattoirs (Manyi-Loh & Lues, 2023). The presence of diverse resistance genes in ExPEC isolates from abattoirs pose a significant public health risks increasing meat contamination leading to foodborne diseases that are difficult to treat, eventually contributing to the spread of AMR and more expensive complex treatment (Guragain et al., 2025). This highlights the need for control measures to alleviate the spread of antibiotic resistance in abattoirs and food chain.

5.4 The presence of virulence genes in Extraintestinal pathogenic *Escherichia coli* isolates and designation of slaughter

Among the isolates, seven virulence genes, namely, *ompA* (100%), *mat* (83%), *kpsm* (65%), both *csg* and *sitA* (57%), *sat* and *traT* (52%) were detected at a significantly greater rate in ExPEC isolates. The findings of this research are supported by the findings reported from APEC isolated in China with high prevalence of *ompA* and *fimC* (Dou et al., 2016), as well as findings from Aziz et al., (2022) in broiler chickens. The *ompA* is a gene that encodes a protein with amino acid variant contributing to facilitates the intracellular survival of *E. coli* strains and safeguards them against the host's defence mechanism (Sonola et al., 2022), it contributes to ExPECs ability to resist serum-mediated killing allowing the bacteria to survive in the bloodstream. It also aids NMEC's to cross the blood–brain barrier, and it has a potential vaccine target for ExPEC, potentially influencing strain virulence and vaccine efficiency (Nielsen et al., 2020). In addition, *OmpA* plays a crucial role in the adhesion of leukocytes and macrophages to pathogenic *E. coli* strains (Sora et al., 2021). Further findings may be useful in SA to investigate the prevalence of ExPEC for the vaccination control of NMEC in broiler chickens.

KpsM is a component of the gene cluster that produces capsular polysaccharide, a key virulence factor in these bacteria that can harm the host immune system, making it more difficult for the host to clear the infection, the capsule helps the ExPEC to resist complement mediated killing and phagocytosis (Zong et al., 2016). Jakobsen et al. (2010) reported lower prevalence of *kpsM* II (23%) from imported meat among broiler chicken isolates in comparable to this study. It is the predominant gene that may result in bloodstream infections, particularly meningitis. The *mat* gene also plays a role in meningitis associated and temperature regulated fimbriae (Pouttu et al., 2001; Sora et al., 2021). The high rate of *mat* gene raises concerns as this gene is rarely isolated *in E. coli* from chicken and in SA, additional research is required to confirm its prevalence and the significance virulence it causes in *E. coli*.

The study found that formal abattoir-sourced isolates had the highest number of virulence genes compared to informal abattoir and freeze-dried isolates, however, this difference was not statistically significant. A higher number of virulence genes in bacteria is associated with increased potential to cause severe infections. The bacteria have DNA that aids in host infection, immune evasion, and damaging human defences (Chen et al., 2025). Formal

abattoirs, despite improved hygiene, can still harbour more virulent genes due to selective pressure in controlled environments, favouring the survival and spread of ExPEC (Zou et al., 2021). The presence of the genes in the *E. coli* isolates underscore the importance of proper chicken meat handling and processing practices to minimize the risk of foodborne diseases.

5.5 Analysis of phylogenetic distribution and virulence genes in Extraintestinal pathogenic *Escherichia coli* isolates and designations

The most prevalent phylogenetic groups in chickens are A1 and B1, followed by B2 and D (Obeng et al., 2012). Characterizing phylogenetic groupings is of clinical significance as previous studies show that commensal *E. coli* strains belong to group A and B1, while pathogenic strains causing extraintestinal infections primarily belong to group B2 and comparatively less to group D (Saralaya et al., 2015). This study showed that phylogroups A followed by B2 were the most frequent phylogroups of *E. coli* obtained from chicken carcasses, which is in agreement with the results reported by (Montes-Robledo et al., 2023) with isolates classified into four phylogenetic groups: A (48%), B2 (26%), D (19%), and B1 (7%). Coura et al. (2017) also supports the findings of phylogroup A being the most prevalent in broiler chickens. This suggests that systematic contamination occurred primarily from phylogroup A and to a lesser extent, group B1. According to Pinheiro et al., (2021), commensal *E. coli* is the cause of chicken carcass infection. This observation is supported by the fact that phylogroup A was isolated from both formal and informal abattoirs. In addition, phylogroup B2 being the second highest rate supports the suggestions that group B2 is prevalent in ExPEC isolates and comparatively less to group D. Overall, these results show that phylogroups B1 and D are not common ExPEC phylogroups isolated from broiler chickens. In SA, most commensal *E. coli* found in chicken meat originates from phylogroup A, indicating a prevalence of intestinal-associated strains in broiler carcasses, unlike the pathogenic strains (group B2/D) found in developed countries (Malesa et al., 2024). Research has primarily focused on phenotypic traits, especially MDR profiles, while molecular phylogenetic distribution has been less studied compared to developed countries. A recent study noted that 91.8% of *E. coli* isolates from chicken and live bird markets display significant MDR, but there is uncertainty regarding their origins, whether from high-pathogenicity B2 strains or commensal B1/A strains that acquired resistance genes (Aworh et al., 2021). The study findings suggests that more research need to be carried out in SA to rule out the occurrence rate of ExPEC phylogenetic grouping in chicken

carcasses and their associated abattoirs as majority of studies done in developed countries have the highest prevalence of group B2 and Group D.

5.6 Presence of integrons in Extraintestinal pathogenic *Escherichia coli*

The findings of the study support the fact that integrons are common in *E. coli* isolates from chicken abattoirs (Wu et al., 2015). Thirty-five percent of the total number of isolates were found to carry *intI1*. These findings align with the findings from Cocchi et al. (2007) with 37% and Patel et al., (2024) with 32% isolated from broiler chickens. Nine percent of the total number of isolates were found to carry *intI2*. These results are notably at a lower rate compared to *intI1* results; this statement is supported by finding from Cavicchio et al. (2015) and Wu et al. (2015) reporting the prevalence of *E. coli* in poultry isolates where *intI1* is occurrence is higher than *intI2*. The study found that *intI3* had the highest occurrence rate of 74% in chicken carcasses, an unanticipated finding given its typically non-isolated nature in chickens and their abattoirs. Findings on *intI3* is primarily reported from clinical isolates from the region of Iran (Kaushik et al., 2018), this is also supported by the finding of Kargar et al. (2014) reporting 26% of multi drug resistance isolates from Iran. There are very few studies that mentions the presence of *intI3* in *E. coli*, especially ExPEC isolates in SA. The fact that *intI3* had the highest rate and typically co-occur with *intI1* and *intI2* in multidrug resistance isolates, it gives more evidence that *intI3* could be crucial in the future spread of antibiotic resistance.

5.7 Distribution of pathotyping Extraintestinal pathogenic *Escherichia coli*

The distribution among the various pathotypes were fairly equal amongst the isolates although the lowest pathotype was NMEC. Contrary to this data, isolates of ExPEC pathotype from chickens vary from study to study. For example, Meena et al. (2021) reported SEPEC at 47%, APEC at 35%, UPEC at 20%, and NMEC at 2%. A study in the USA found higher percentage of SEPEC (55%), APEC (40%), NMEC (11%), but a lower prevalence of UPEC (Mitchell et al., 2015) while a similar prevalence was reported in Canada (Stromberg et al., 2017). Variations in sample sources or geographic locations may account for the variations in ExPEC pathotype prevalence rates observed.

5.8 Concluding remarks

This research highlights food safety concern in Gauteng, revealing a 93% prevalence of *E. coli* in chicken carcasses due to poor sanitation in abattoirs, as well as 96% prevalence of IPEC. Poultry is a significant reservoir for pathogenic *E. coli*, increasing risks of foodborne diseases. The extensive use of antibiotics in the poultry sector has led to high rates of antimicrobial resistance, including 57% resistance to tetracycline and notable resistance to ciprofloxacin. The formal abattoirs exhibited higher antimicrobial resistance (58%). The emergence of multi-drug resistance ExPEC in poultry endangers human antibiotic efficacy. Although no isolates carried both *tetA* and *tetB* genes, indicating a limited resistance profile, monitoring is essential as these genes represent resistance mechanisms. The findings indicate a high prevalence of key virulence genes (*ompA, mat, kpsm, csg, sitA, sat, traT*) in ExPEC isolates, suggesting enhanced survival capabilities in the bloodstream and evasion of host immune defences mechanism. Notably, the 100% prevalence of *ompA* gene indicates its function in serum-mediated cytotoxic resistance, intracellular survival, and potentially bridging the BBB. The study reveals that phylogroup A is the most common *E. coli* group found in chicken carcasses, succeeded by B2. The prevalence of *intI1* and *intI2* integrons is consistent with existing literature, but the unexpected predominance of *intI3* at 74% indicates a growing genetic trend in South African chicken isolates that may have a major impact on the future spread of multidrug resistance.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study showed that *E. coli* levels in chicken meat from both formal and informal chicken abattoir were detected in a significant proportion especially considering the various geographical locations from Gauteng municipalities. Furthermore, the presence of ExPEC in chicken meat were significantly higher in formal abattoirs compared to informal abattoirs. The study indicates ongoing hygiene challenges in chicken meat, despite stricter regulations. The findings suggest risks of meat contamination and the potential spread of pathogenic *E. coli* to humans through the food chain and environment, along with implications for animal welfare and production.

The study underscores the necessity of monitoring microbiological quality in both formal and informal markets, as ExPEC strains can harbour antibiotic-resistance bacteria, potentially affecting human health. Extraintestinal pathogenic *E. coli* isolates exhibited varying levels of resistance to different antibiotics as well as the resistance genes, highlighting the need for cautious use of antimicrobial in poultry production. The presence of the highest rate of *intI3* in the isolates suggest the potential mechanism for the acquisition and dissemination of antibiotic resistance.

The study revealed virulence genes and integrons in ExPEC, underscoring potential health risks and the need for monitoring and controlling antibiotic resistance in poultry. It goes without saying that isolates from formal abattoir had the highest levels of phenotypic and genotypic resistance. The study detected the range of virulent genes especially *ompA*, potentially could contribute to the pathogenicity of the ExPEC resulting to the development of ExPEC infection. The presence of the variety of virulent genes in abattoirs highlights the potential risk of food borne transmission of pathogenic *E. coli* to human.

The ExPEC isolates of this study predominantly belonged to phylogenetic groups B2 and A. The data indicates a strong correlation between ExPEC strains and the causes of UTIs and septicemia. However, statistical analysis found no significant link amongst the distribution of

pathotypes and strain designations, even in isolates with the lowest prevalence of ExPEC pathotypes.

6.2 Recommendation

This study revealed the high prevalence of contamination level in formal abattoirs suggesting the need for continuous stricter implementation of abattoir biosecurity protocol and antibiotic use protocol in chicken farms involving veterinarians that can aid to reduce *E. coli* infections.

It is important to encourage chicken farmers to sparely use antibiotics in poultry production to reduce the risk of antibiotic resistance. Veterinarians are encouraged to incorporate systematic field-level surveillance of AMR into their standard clinical protocols. Department of Agriculture, Land Reform and Rural Development, veterinarians, Department of health and researchers should establish a surveillance system to produce monitor the prevalence of ExPEC and antibiotic resistance in chickens. South African Poultry Association in collaboration with Department of Agriculture (veterinary services) should establish guideline for best practices in chicken production to minimize the risk of ExPEC contamination.

There is also a need to encourage consumers of proper handling (cooking) of chickens and the risk of food borne illnesses when purchasing chickens from informal abattoirs. The initiative aims to promote collaboration between abattoirs, the poultry sector, and public health authorities to exchange knowledge and develop best practices that can be used for prevention of bacterial infections.

6.3 Future studies

- Future research ought to investigate the prevalence of ExPEC in various species considering that there aren't adequate studies on the subject matter of food security in SA.
- The study was geographically limited to Gauteng Province, suggesting that future research should consider a wider geographical scope within the different provinces in SA.
- Future research should consider employing whole genome sequencing to look at the holistic molecular characterization considering plasmids, serotyping of ExPEC.
- Conduct research to comprehend the genetic diversity and pattern of transmission of ExPEC in chicken abattoirs.

6.4 Limitations

The study is subject to limitations outlined:

- The study's comprehensive characterization of ExPEC isolates was limited by the inability to perform serotyping, which was restricted due to budget constraints. The inability to perform serotyping hinders scientific analysis and virulence characterization of ExPEC strains, limiting the tracking of outbreaks and understanding of transmission dynamics, particularly in linking isolates between abattoirs and chicken meat.
- The study was unable to proceed with the characterisation of Enterobacterial Repetitive Intergenic Consensus PCR because of the challenges associated with data analysis following data collection, given that the facility lacked the skilled personnel to carry out this duty. The implications of this is the inability to differentiate ExPEC strains effectively and the shortage of linking isolates with the source (chicken).

CHAPTER 7: REFERENCE

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APPENDIX

Thermo SCIENTIFIC

SENSITITRE™ GRAM NEGATIVE NARMS PLATE FORMAT

Plate Code: **CMV3AGNF**

	1	2	3	4	5	6	7	8	9	10	11	12
A	FOX 32	AZI 8	CHL 16	AXO 64	AXO 0.25	CIP 2	GEN 16	NAL 16	XNL 2	FIS 32	AMP 32	STR 16
B	FOX 16	AZI 4	CHL 8	AXO 32	AUG2 32/16	CIP 1	GEN 8	NAL 8	XNL 1	FIS 16	AMP 16	STR 8
C	FOX 8	AZI 2	CHL 4	AXO 16	AUG2 16/8	CIP 0.5	GEN 4	NAL 4	XNL 0.5	SXT 4/76	AMP 8	STR 4
D	FOX 4	AZI 1	CHL 2	AXO 8	AUG2 8/4	CIP 0.25	GEN 2	NAL 2	XNL 0.25	SXT 2/38	AMP 4	STR 2
E	FOX 2	AZI 0.5	TET 32	AXO 4	AUG2 4/2	CIP 0.12	GEN 1	NAL 1	XNL 0.12	SXT 1/19	AMP 2	NEG
F	FOX 1	AZI 0.25	TET 16	AXO 2	AUG2 2/1	CIP 0.06	GEN 0.5	NAL 0.5	FIS 256	SXT 0.5/9.5	AMP 1	POS
G	FOX 0.5	AZI 0.12	TET 8	AXO 1	AUG2 1/0.5	CIP 0.03	GEN 0.25	XNL 8	FIS 128	SXT 0.25/4.75	STR 64	POS
H	AZI 16	CHL 32	TET 4	AXO 0.5	CIP 4	CIP 0.015	NAL 32	XNL 4	FIS 64	SXT 0.12/2.38	STR 32	POS

ANTIMICROBICS

FOX	Cefoxitin
AZI	Azithromycin
CHL	Chloramphenicol
TET	Tetracycline
AXO	Ceftriaxone
AUG2	Amoxicillin / clavulanic acid 2:1 ratio
CIP	Ciprofloxacin
GEN	Gentamicin
NAL	Nalidixic Acid
XNL	Ceftiofur
FIS	Sulfisoxazole
SXT	Trimethoprim / sulfamethoxazole
AMP	Ampicillin
STR	Streptomycin
NEG	Negative Control
POS	Positive Control

Figure 20: Customized Gram negative Sensititre™ 96-well microtiter plate.

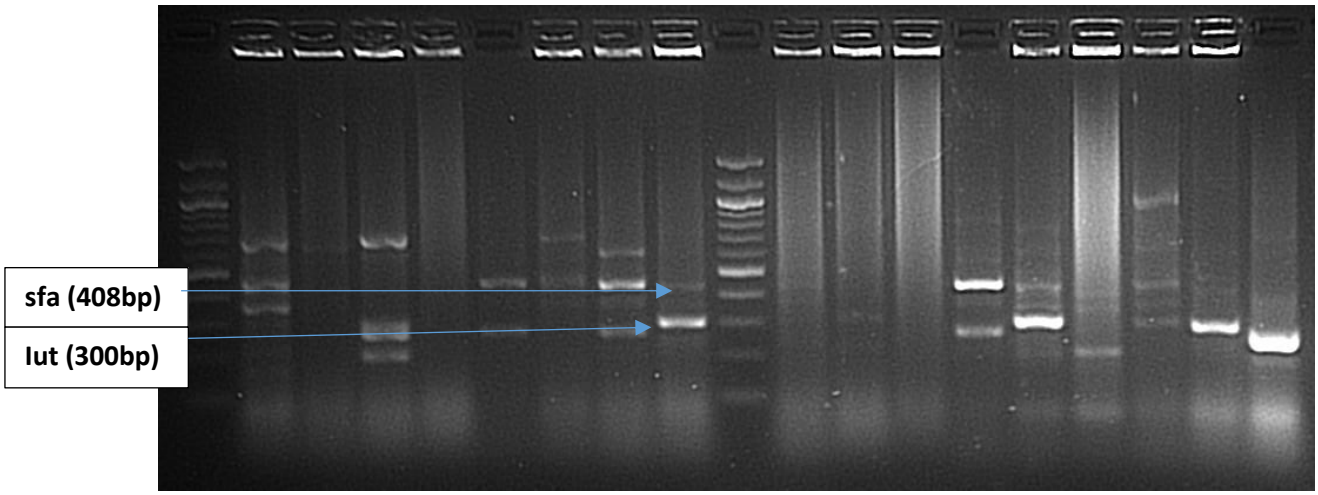

Figure 21: EXPEC PCR Gel electrophoresis results

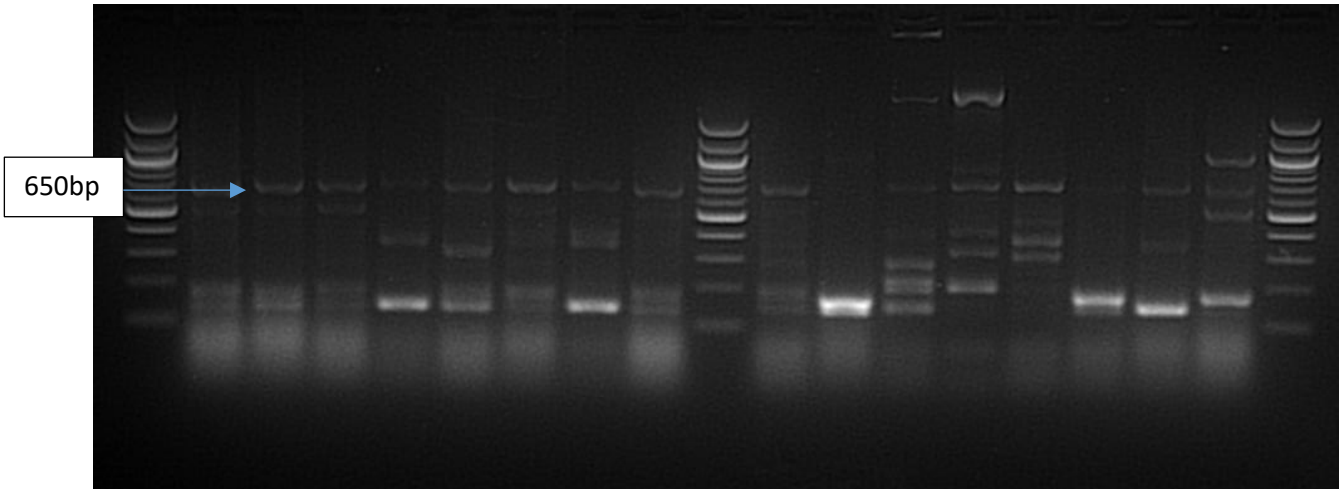

Figure 22: Integron 3 Polymerase Chain Reaction Gel electrophoresis results

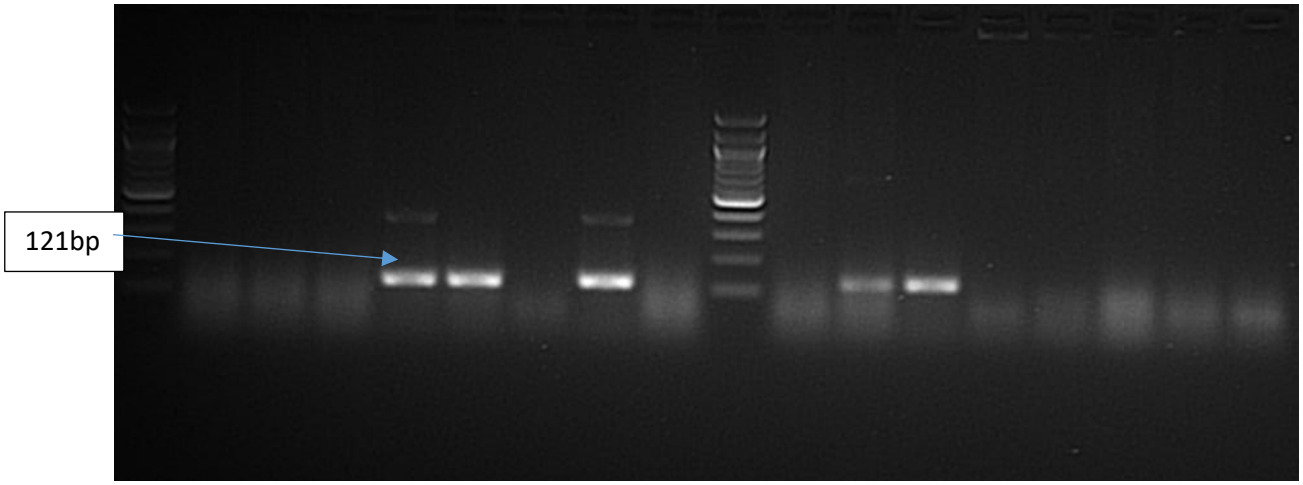

Figure 23: *chuA* Polymerase Chain Reaction Gel electrophoresis results

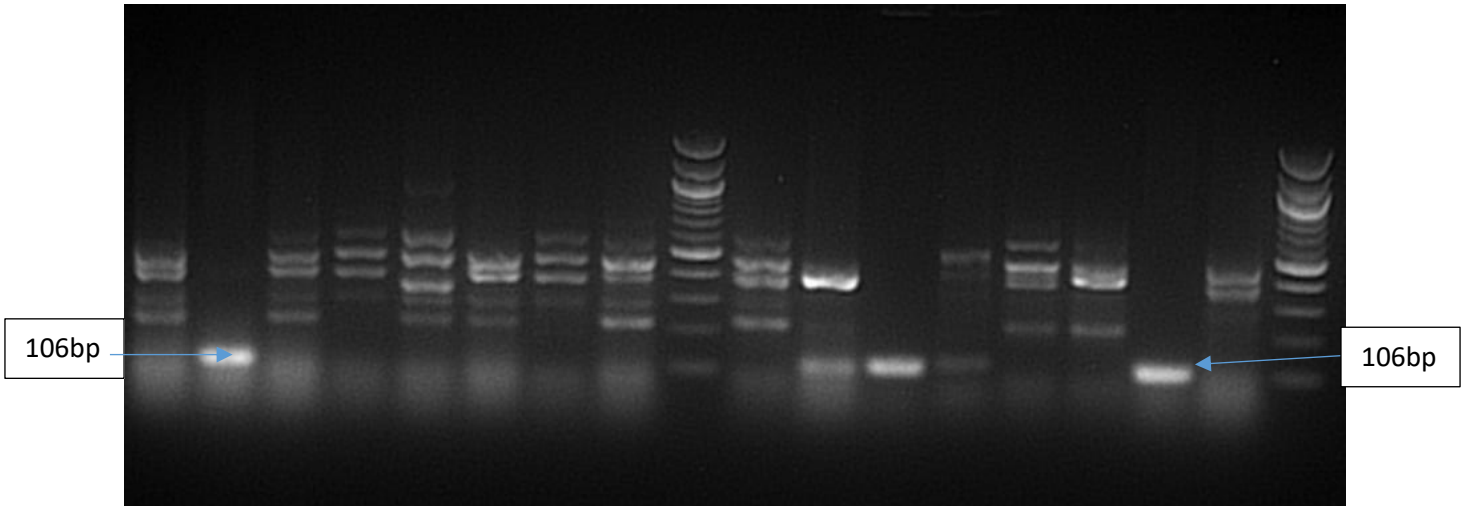

Figure 24: Phylogenetic group gene *yjaA* Polymerase Chain Reaction Gel electrophoresis results

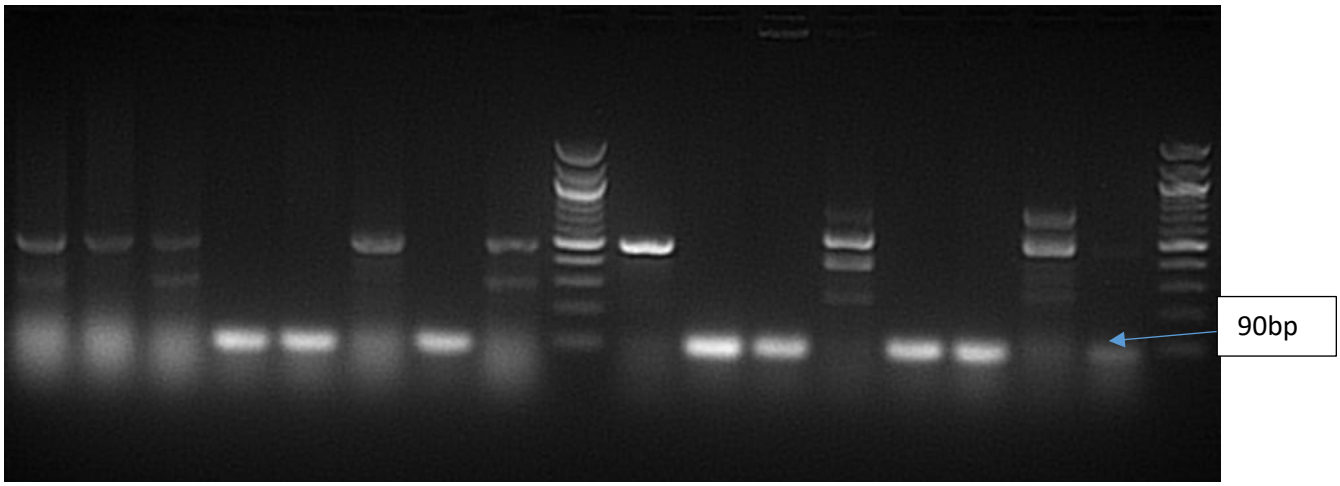

Figure 25: *TPSE*4.C2 Polymerase Chain Reaction Gel electrophoresis results