An-Najah National University

Faculty of Graduate Studies

ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* ISOLATES RECOVERED FROM BROILERS' FARMS IN NORTHERN PALESTINE

By

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Dedication

The researcher may dedicate his work to relatives, friends and family members.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* ISOLATES RECOVERED FROM BROILERS' FARMS IN NORTHERN PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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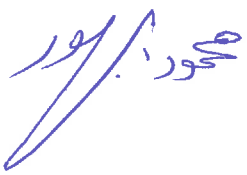
Signature: 

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Abstract

Background: Colibacillosis is a disease caused by a certain type of *Escherichia coli* called the avian pathogenic *Escherichia coli* (APEC), which leads to significant losses for the poultry sector and shows zoonotic potential and acts as a source of antibiotic resistance and virulence factors for other *E. coli*.

Objectives: This work aimed to assess resistance phenotype, virulence genes, and phylogenetic groups in APEC isolates recovered from broilers' farms in northern Palestine. As well as to clarify whether the virulence factors are directly associated with antibiotic resistance or, instead, dependent on a phylogenetic group distribution.

Methodology: A total of the 65 APEC isolates were recovered from diseased chicken with typical colibacillosis symptoms from broilers' farms located in the northern region of Palestine, during the period from May to July 2024. Classical and molecular techniques were used to identify these strains. The disk diffusion method was used to detect antibiotic resistance. Phylotyping and virulence genotyping of these APEC isolates were carried out by polymerase chain reaction.

Results: This study revealed a high detection rate of APEC strains (100%) in chicken. The most APEC strains 56/65 (86.2%) assigned to group D. Other strains were related to groups B2 (5/65, 7.7%), B1 (3/65, 4.6%) and A (1/65, 1.5%). Antibiotic resistance ranged from 27.7% for PolymyxinsE (Colistin) to 100% for Amoxicillin. Polymyxins E (Colistin) and Fosfomycin were the most effective drugs. It was found that the most common virulence factor was *iroN* which was tested in 61 isolates (93.8%). While, 56 (86.2%), 42 (64.6%), 40 (61.5%), 37 (56.9%), 24 (36.9%), 23 (35.4%),16 (24.6%), 13

(20.0%), 0 (0.0%) and 0 (0.0%) isolates were positive for *hlyF, iutA, Tsh, ColV, papGII, Iss, papGI, papC, papGIII* and *ompT* genes, respectively. The APEC strains in Palestine exhibit a wide variety of resistance patterns and genetic variation.

Conclusion: These results serve as an outline for development of efficient intervention plans for the management of APEC in broiler breeders and broiler farms. Controlling APEC infections is essential for public health, especially when APEC isolates can pass on virulence and resistance factors to other pathogenic bacteria such as *E. coli* that are particular to humans.

Keywords: APEC, Colibacillosis, Virulence factors, Antibiotic resistance, Phylogenetic group, Palestine.

Chapter One
Introduction

1.1 Theoretical basis

1.1.1 *Escherichia coli* and avian pathogenic *E. coli* (APEC)

The species *E.coli* commonly referred to as *E. coli*, belongs to the Enterobacteriaceae family of commensal bacteria. Bacteria of this species are rod-shaped, Gram-negative, lactose, catalase, and indole-positive, but negative for oxidase, urease, and citrate. *E.coli* can receive particular pathogenic factors and become linked to a range of animal illnesses. These particular features, along with the site of infection, have allowed for the classification of these *E. coli* strains into distinct pathotypes. It is thought to be one of the primary causes of foodborne infections. It is frequently found in the digestive system of warm-blooded animals, humans, and poultry.

E. coli occurs frequently in the flora of the gastrointestinal tracts of animals and humans, where it symbiotically participates in the production of vitamins and digestion. There are already approximately 160 serological forms of *E. coli*, and somatic (O) 171 serotypes, flagellar (H) 55 serotypes, and capsular (K) antigens 80 serotypes have been detected. Meningitis, sepsis, hemolytic-uremic syndrome (HUS), urinary tract infection (UTI), hospital-acquired pneumonia (HAP) and surgical site infection (SSI) are all associated with *E. coli* (Alkeskas et al., 2015).

Three primary types of *E. coli* strains have been identified: intestinal pathogenic or diarrheagenic *E. coli* strains (DEC), extraintestinal pathogenic *E. coli* (ExPEC) strains, and commensal strains. One of the most important bacteria that may lead to diarrhea is DEC, a bacterium that can also cause gastroenteritis. The six pathotypes of DEC are as follows: diffusely adherence *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC), enterohemoragic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC). The DAEC, ETEC and EPEC pathotypes influence the small intestine, while the other pathotypes affect the colon (Miri et al., 2017).

The avian pathogenic *E. coli* (APEC), uropathogenic *E. coli* (UPEC), newborn meningitis *E. coli* (NMEC) and sepsis associated *E. coli* (SEPEC) subgroups or subpathotypes make up the ExPEC category (Afayibo et al., 2022). A subpathotype of ExPEC called APEC is

to blame for several systemic and local diseases in birds, especially in ducks, chicken, turkeys, and many other species. Various bird organs can become infected with APEC. APEC is also one of the frequent bacterial diseases causing mortality and morbidity in geese and ducks, raising concerns in China's waterfowl breeding industry, which produces 20-30 billion ducks each year. Game birds, geese and wild ducks can carry APEC and represent possible transmission vectors between reservoirs. APEC therefore represents a worldwide danger to food safety and avian welfare (Mehat et al., 2021). APEC has always been considered as a secondary pathogenic organism, after Mycoplasma or viral infection. In the last 10 years, APEC has been considered the major pathogen in avian hosts that causes colibacillosis, which is a syndrome characterized by a variety of generalized and localized infections (Mehat et al., 2021). Among the most common lesions are omphalitis, salpingitis, cellulitis, pericarditis, airsacculitis, perihepatitis, pericarditis, coligranuloma, egg peritonitis, and systemic infections. According to Kathayat et al., (2021), these extraintestinal conditions are known as avian colibacillosis. They cause in chicken a high rate of mortality and morbidity, as well as decreased production of meat and eggs, a lower rate of hatching, and a higher rate of carcasses being condemned at slaughter (Azam et al., 2019; Kathayat et al., 2021; Bhattarai et al., 2023). This represents economic and financial losses to the worldwide chicken industry (Johar et al., 2021; Bhattarai et al., 2023).

In addition to the aerosol or fecal-oral routes, contaminated food and water are the usual ways that chicken get the disease. Furthermore, APEC can move vertically from ill breeders to commercial day-old chicks via contaminated eggs. This gives the poultry industry serious cause for concern. Moreover, APEC is common in all age categories of chicken (9.5-36.7%) (Bhattarai et al., 2023).

Young birds are more vulnerable to APEC; they are typically broiler chicken between the ages of 28 and 42 days, whereas layers are affected at every stage of development (Bhattarai et al., 2023). The APEC uses different types of pathogenesis and virulence genes that participate and promote the development of disease in chicken, including invasins, adhesins, iron acquisition systems, toxins and protectins. These factors promote APEC proliferation, evasion from the host immunological responses, adhesion, invasion, colonization, and systemic transmission, permitting the development of infection in chicken. Other bacterial virulence and pathogenesis factors include secretion systems,

metabolism-associated genes, transcriptional regulators, quorum-sensing systems, two-component systems, genes essential for systemic infections, and adaptation and miscellaneous (Kathayat et al., 2021; Joseph et al., 2023).

Some studies showed that certain human ExPEC strains and APEC strains share common phylogenic backgrounds and share some similar virulence genes and these strains can infect humans (Sarowska et al., 2019; Johar et al., 2021). Furthermore, similarities have been observed between the APEC strains in boilers and ExPEC in humans; suggesting there is a high likelihood of horizontal gene transfer between APEC and human ExPEC strains (Johar et al., 2021). Analysis of APEC strain genomes showed that may be APEC strains are a reservoir for human-pathogenic ExPEC virulence genes.

Ten major virulence genes (*iutA*, *iucC*, *cvaC*, *iucD*, *cvaB*, *cvaA*, *cvi*, *ompT*, *hlyF*, and *etsA*) shared by avian-associated ColV plasmids in human ExPECs and APECs indicate to potential transmission of APEC from poultry to people (Rodriguez-Siek et al., 2005; Ewers et al., 2007; Johnson et al., 2008). The health of humans and animals may benefit from the management of avian colibacilloses. In mouse models, APEC isolates can cause meningitis and urinary tract infections (UTI) (Jakobsen et al., 2010; Tivendale et al., 2010). Many virulence genes that are encoded on bacteriophages, plasmids, or inside pathogenicity islands on the bacterial chromosome and other mobile elements are present in *E. coli* strains that cause illnesses (Bhattarai et al., 2023).

It is accepted that pathogenic strains of *E. coli* may have acquired virulence operons from nonpathogenic strains through chromosomal or extrachromosomal transfer (Branger et al., 2005). Several studies have shown that some virulence factors encoded by diverse genes enhance APEC pathogenicity to cause colibacillosis and to grow in the tissues of broilers (Azam et al., 2019; (Afayibo et al., 2022). The traditional diagnostic technique used in laboratories to identify *E. coli* linked to colibacillosis involves serotyping APEC strains based on somatic O antigen identification, using antisera or PCR technique (Mehat et al., 2021). Moreover, APEC isolates belong commonly to serotypes O1, O2, O8, O15, O18, O35, O36, O78, O88, O109, O111, and O115. Among the 188 O-antigen serotypes identified for APEC, O78, O2, and O1 are widely distributed and are thought to represent 80% of APEC isolates phylogroup globally and are most associated with colibacillosis (Rodriguez-Siek et al., 2005; Mehat et al., 2021; Fancher et al., 2021; Bhattarai et al., 2023).

Serotyping is still the most commonly diagnostic method used in laboratories, although its application to distinguishing specific APEC strains is limited. Serotype fails to show virulence traits; hence this method is ineffective as a diagnostic tool. Numerous studies have emphasized the concept of using virulence factors to differentiate among APEC strains. Nevertheless, only a limited proportion of APEC isolates can be classified using the serotyping approach. The approach that is most commonly used for identifying and classifying the avian colibacillosis causal agent is the PCR-typing method, which targets 10–15 virulence genes and designates isolates with more than 4 virulence determinants as APEC. These isolates are obtained from hens that exhibit particular clinical signs or symptoms (Kim et al., 2020; Mehat et al., 2021).

Several studies have tried to determine the virulence determinants linked with APEC strains; these major markers include virulence factors, such as *cva/cvi, papC, hlyE/F, iucD, iroN, irp2, iutA, iss, ompT,* and *tsh* amongst others (Subedi et al., 2018; Cummins et al., 2019). In other studies, the main predominant virulence genes found in APEC implicated in avian colibacillosis include *hlyF*, *iss*, *fimH*, *iutA*, *iroN*, *ompT*, *kpsMTII*, *iucD*, *traT*, *sat chro*., *tsh*, *cvi/cva*, and *aerJ* (Silveira et al., 2016; Azam et al., 2019; De Oliveira et al., 2020). Good and effective biosecurity measures along with decontamination of feed and water sources and disinfection of poultry houses and farm equipment inside and out are essential to prevent the entry of APEC onto farms (Dho-Moulin & Fairbrother, 1999; Dziva & Stevens, 2008).

Controlling environmental stressors such as ammonia and air in poultry houses by maintaining a good level of litter and air is one of the main factors in preventing APEC infection in poultry farming. Furthermore, controlling the causative agents through chicken vaccination programs against Mycoplasma galliseptica, infectious bronchitis, Newcastle disease, and infectious bursal disease reduces the incidence of APEC infections (Dho-Moulin & Fairbrother., 1999;Dziva & Stevens, 2008). Vertical transmission of APEC infection can be prevented through several methods, like developing strains with increased resistance to APEC infection, constant attention to cleaning and disinfecting eggs inside the incubator, and limiting the use of ground eggs (Christensen et al., 2021).

1.1.2 Pathogenesis and Virulence Factors on APEC

APEC causes colibacillosis in chicken, either it has or uses various virulence and pathogenesis mechanisms or determinants. These determinants or factors include invasins, two-component systems, adhesins, toxins, protectins, secretion systems, iron acquisition systems, genes related to metabolism, quorum-sensing systems, and transcriptional regulators. All these genes play different roles in APEC infections, which include invasion of the host cells, attachment to host cells, cell lysis and damage, colonization of tissues, bloodstream persistence, survival inside phagocytic cells, replication and proliferation in cells, resistance to oxidative and environmental stresses, motility, biofilm formation and sequestering metals from bodily fluids for growth (Kathayat et al., 2021).

1.1.2.1 Adhesins

Adhesins are cell-surface components or bacterial appendages that aid in attachment to surfaces on the host cells. Adherence is a crucial stage in the infection process or bacterial pathogenesis and is necessary for colonization. Type P fimbriae, type S fimbriae and type 1 fimbriae are the main factors that enhance adherence in APEC. A number of genes, including *papG I, papG II, papGIII, papC, papEF, papA, tsh* (temperature-sensitive haemagglutinin), and others, encode these fimbriae and other adhesins. Additionally, certain adhesins facilitate intracellular survival, biofilm formation, colonization, motility and stress tolerance in APEC (Kathayat et al., 2021).

1.1.2.2 Invasins

A family of proteins known as invasins is linked to the entrance of infections into host cells. throughout the early stages of the infection, invasins have a function in encouraging entrance. APEC isolates have been shown to harbor many factors encoding invasins, *ibeB* (invasion protein), *ibeA* (also known as ibe10), *gimB* (genetic island linked with newborn meningitis) and *tia* (toxigenic invasion locus). Furthermore, invasins support APEC proliferation in host cells, expression of virulence genes, colonization, biofilm formation, oxidative stress generated by macrophages, invasion, expression of membrane proteins, metabolism genes, resistance to environmental and serum stresses, motility and adhesion (Kathayat et al., 2021).

1.1.2.3 Iron Acquisition Systems

Once bacteria have effectively colonized and/or invaded the host, iron is a crucial micronutrient needed for the development and multiplication of the bacteria inside the host. To sequester iron from bodily fluids, APEC has many iron acquisition systems that include transporters and several siderophores, including yersiniabactin, salmochelin, and aerobactin. In APEC, there are multiple genes responsible for encoding the iron uptake and transport systems, including *mntH* (iron and manganese transporter), *sitABCD*, *bfr* (bacterioferritin), *ireA* (iron-regulated virulence gene), *feoB* (ferrous ion transporter), *fyuA* (yersiniabactin), *irp2* (iron repressible protein), *chuA* (outer membrane hemin receptor), *eitABCD* (putative iron transporter), fepC (ferric enterobactin transporter), iutA, *iucCD*, *aerJ* (aerobactin), and *iroBCDEN* (salmochelin). Furthermore, these siderophores and transporters influence the expression of additional virulence determinants, resistance to environmental stresses, invasion, persistence in the host, adhesion, as well as APEC colonization. Moreover, the genes encoding the enterobactin synthesis, outer membrane efflux protein (*tolC*), and transport genes (*entE* and *entS*) work together to promote invasion, colonization and persistence (Kathayat et al., 2021).

1.1.2.4 Protectins

Protectins protect bacteria against a variety of adverse environments and the immune system of the host. Protectins, in particular, are components of lipopolysaccharide (LPS), outer membrane proteins, and bacterial capsules. They offer defense against bactericidal action mediated by complement in the host serum as well as protection against macrophage phagocytic engulfment. Multiple genes encoding protectins, *kfiC-K5* (glycosyl transferase), *iss* (increased serum survival), *ompT* (outer membrane protease), traT (complement resistance protein), beta (choline dehydrogenase), *kpsMT(K1)*, *kpsMT(II)*, *kpsMT(III)*, *neuC*, *neuS* and *neuD* (capsule) are among the genes that have been detected in APEC. Furthermore, to mediate protection from host defense, these protectins also facilitate APEC colonization, adherence, proliferation in the host, intracellular survival and invasion. Additionally, involved in invasion, the outer membrane proteins *PagP* and *YbjX* , play a role in tolerance to environmental and serum stresses and intracellular survival. In the same way, another outer membrane protein (*OmpA*) helps APEC isolates survival and existing in macrophages. The genes involved in the biosynthesis of LPS, *wzy* (O-antigen polymerase) and *waaL* (O-antigen ligase),

promote motility, colonization, biofilm formation, invasion, adhesion, resistance to phagocytosis, environmental stresses and intracellular survival. Similarly, colonization, intracellular survival, invasion and the control of nitric oxide generation and cytokine gene expression are all impacted by the lipid A biosynthesis gene *lpxM* (myristoyl transferase). On the other hand, superoxide dismutase, or *sodA*, promotes the development of biofilms and protects APEC from reactive oxygen species (ROS) mediated by host defense (Kathayat et al., 2021).

1.1.2.5 Toxins

Toxins refer to biological molecules that facilitate bacterial invasion and subsequent tissue damage. Several genes encoding toxins, *pic* (serine protease autotransporter*)*, *EAST-1*, *AstA*, (heat-stable enterotoxin), *ace4/35* (acetylcholine esterase), *espC* (serine protease), *hlyF*, *hlyA*, *hlyE* (putative avian hemolysin), *stx2f* (Shiga toxin variant), *vat* (vacuolating autotransporter toxin), *sat* (secreted autotransporter toxin), *cdtS* and c*dtB* (cytolethal distending factor), have all been reported in APEC. Additionally, these toxins promote the induction of vacuolization, agglutination, biofilm formation, motility and colonization (Kathayat et al., 2021)**.**

1.1.2.6 Other Virulence and Pathogenesis Factors

The transcriptional regulators, secretion systems, two-component systems, genes linked to bacterial metabolism and QS system are additional virulence and pathogenicity components of APEC. These factors facilitate the proliferation of APEC and the establishment of disease in the host by supporting various processes of APEC pathogenesis/infection, such as modulation of host immune responses, interbacterial competitions, invasion, persistence, colonization, resistance to host defenses, and adhesion (Kathayat et al., 2021).

1.1.2.6.1 Quorum-Sensing (QS) System

In bacteria, quorum sensing is a mechanism of cell-to-cell communication based on autoinducers. It controls the expression of many genes associated with the development of biofilms, pathogenicity, motility and others. The *Lsr* operon controls the activity of the LuxS-synthesised autoinducer-2 (AI-2) molecule, which mediates QS in APEC. The phosphotransferase system (ptsI), the *AI-2* operon, the LuxS, the *Lsr* operon, and the activated methyl cycle pathway (Pfs) all aid in the pathogenesis of APEC through

different mechanisms, including colonization, biofilm formation, invasion, motility, intracellular survival, invasion, adherence, persistence, cell damage and expression of virulence genes (Kathayat et al., 2021).

1.1.2.6.2 Secretion Systems

These are cell-associated systems present on bacterial cell membranes that secrete proteins into the host cells, leading to destroy those cells. The released proteins increase bacterial pathogenicity by directly by improving adhesion to the host cells or intoxicating the host cells, creating a niche for replication by scavenging resources and competing with other microbes. There are two secretion systems called type III and VI, involved in the pathophysiology of APEC. Adhesion, intracellular survival, Motility, proliferation, colonization, downregulation of pro-inflammatory cytokine responses, resistance to phagocytosis and serum bactericidal activity, and the expression of fimbriae genes are all controlled by the regulators ATPase (EivC) of the type III secretion system 2 (ETT2) and (*YqeI* and *EtrA*). Similar to this, various elements of the type VI secretion system, such as *DotU* (organelle trafficking protein), *ClpV* (ATPase), *VrgG* (secreted protein), *CpxA* (envelope stress response system), *IcmF*, *CpxR* (intracellular multiplication factor), and *Hcp* (hemolysis co-regulation protein), mediate the following processes: motility, invasion, intracellular survival, modulation of intracellular host responses (IL-8, IL-1β), production of type 1 fimbriae, interbacterial competition, colonization, adhesion, resistance to serum bactericidal activity and biofilm formation (Kathayat et al., 2021).

1.1.2.6.3 Two-Component Systems

The main signaling proteins in bacteria called two-component systems (TCS), which allow the bacterial genes to change their expression in response to the environment. Various TCSs have been implicated in the pathophysiology of APEC. The expression of virulence genes and genes linked to quorum sensing, flagellar assembly, bacterial ABC transporters, chemotaxis, motility, systemic dissemination, invasion, biofilm formation and adhesion are all influenced by *PhoPQ*, a membrane-associated TCS. Same way, APEC pathogenicity, biofilm formation, and in vivo colonization are facilitated by another membrane-associated TCS, *BasSR*. The expression of resistance to serum bactericidal activity, motility, the formation of flagellum, and flagella-related genes are all mediated by *KdpDE*, a TCS that controls potassium transport. Similar to this, a TCS

regulating nitrogen metabolism, RstAB, helps with intracellular survival, acid resistance, colonization and iron acquisition. BarA-UvrY, a different TCS, is involved in intracellular survival, invasion, persistence, resistance to oxidative stress and expression of type 1 and P fimbriae, serum bactericidal activity, control of exopolysaccharide synthesis, and adhesion (Kathayat et al., 2021).

1.1.2.6.4 Transcriptional Regulators

Several transcriptional regulators have been implicated in the pathophysiology of APEC. Two major global transcriptional regulators, *AutR* and *AutA* control the expression of K1 capsule, acid resistance systems and changes in adaptive lifestyle that promote infection. Another global transcriptional regulator called fumarate and nitrate reduction, or FNR, promotes type 1 fimbriae expression, attachment, invasion, tolerance to oxidative stress and type VI secretion system. While a transcriptional regulator *tyrR*, included in the manufacture and transportation of aromatic amino acids, facilitates intracellular survival and invasion motility. *McbR*, a *MqsR*-controlled colonic acid and biofilm regulator, is implicated in biofilm formation and stress response. On the other hand, a *tyrR* transcriptional regulator, involved in the synthesis and transportation of aromatic amino acids, intracellular survival, promotes motility and invasion. A transcriptional anti-terminator *RfaH*, assists in resistance to serum bactericidal action and invasion and intracellular survival, whereas *YjjQ*, a transcriptional regulator of the *LuxR* family, contributes to the flagellar movement (Kathayat et al., 2021).

1.1.2.6.5 Metabolism-Associated Genes

The pathogenesis of APEC is influenced by many genes linked to bacterial metabolism. The acetate assimilation system is encoded by the operon *acs-yjcH-actP*, which promotes colonization, proliferation, intracellular survival, and the synthesis of nitric oxide and pro-inflammatory cytokines. Adhesion and colonization are similarly mediated by *PotE* (putrescine transporter) and *NirC* (nitrite transporter), which are involved in polyamine production and putrescine transport, and nitrogen metabolism and cytoplasmic detoxification, respectively. Motility and chemotaxis are regulated by the aerobic respiratory control (*ArcA*) gene, which is involved in the transport and metabolism of citrate (Kathayat et al., 2021).

1.1.2.6.6 Miscellaneous

The pathophysiology of APEC is further influenced by several other bacterial elements, including transport systems. porins, putative proteins, genes encoding prophages, enzymes, and Prophages. Prophage phiv205-1 and phiv1423, in particular the *orf20* gene, are involved in adhesion, intracellular survival, resistance to serum and environmental stresses, colonization, biofilm formation, the development of flagella and type 1 fimbriae and invasion. Colonization, invasion, proliferation and Adhesion are facilitated by the outer membrane porins *OmpC* and *OmpF*. A putative protein *YicS*, is involved in invasion, formation of biofilm, and motility. While the phosphate transport system pstSCAB, in particular *pstB*, has a role in oxidative stress and colonization as well as resistance to serum bactericidal activity. Colonization is mediated by cpdB (20, 30-cyclic phosphodiesterase). Resistance to serum bactericidal action is mediated by lysozyme inhibitor *mliC*, whereas persistence, intracellular survival replication, and colonization are mediated by transfer mRNA-small protein B, or *tmRNA-SmpB.* Furthermore, some virulence genes with unclear or unknown activities in APEC have been identified in other bacteria (Kathayat et al., 2021).

1.1.3 Phylotypes of E. coli

The mobile genes of bacteria including *E. coli* can be transferred horizontally between related bacteria or between bacteria from other families, enabling the bacteria to settle in various environmental settings (Sarowska et al., 2019). Previously, it was suggested that *E. coli* collected from human clinical cases and animal hosts could be characterized phylogenetically (Clermont et al., 2013; 2019). Using some particular genes, such as *yjaA*, *chuA*, and *TspE.4C2*, a phylotyping approach was created to classify strains of *E. coli*. At least, eight groups within the species *E. coli* have diverged into three distinct categories within its genetic substructure: phylogenetic groups B2, G, and F, phylogenetic groups A, B1, C, and E, and phylogenetic group D, with group D being the one most closely related to the origins of *E. coli* (Gonzalez-Alba et al., 2019).

Commensal strains with no pathogenic features are more strongly related to groups A and B1 that are associated to environmental reservoirs. Intestinal infections caused by pathogenic *E. coli* belong to phylogenetic groups A, B1, or D. Pathogenic strains that are frequently associated to virulent extra-intestinal infections are more closely related to phylogenetic groups B2 and D, Group F is linked with the primary group B2, whereas

group E is linked with group D (containing O157: H7) (Branger et al., 2005; Luque et al., 2017; J. R. Johnson et al., 2017; Sarowska et al., 2019; Afayibo et al., 2022). As reported by Branger et al., (2005), strains related to phylogenetic groups B2 and D usually possess virulence genes unavailable in bacteria from groups A and B1. Among APEC from septicemia isolates and yolk sac infections, phylogenetic groupings B1 and C were well-known (Afayibo et al., 2022).

Antimicrobial chemotherapy is typically used for treatment APEC infections in chicken and other birds. The Kirby Bauer test (disc diffusion method) is usually employed to assess the sensitivity of the APEC organism *in vitro*, along with the drug's pharmacokinetics and clinical efficacy, to determine which effective antibiotic to utilize. As a result, the selection of antimicrobials available to veterinarians in the poultry sector is limited. Furthermore, inappropriate use of antimicrobial drugs, such as overuse, prophylactic usage, or growth promoter use, might contribute to the increase in antimicrobial resistance rate (Thomrongsuwannakij et al., 2022).

Since pathogenic *E. coli* is believed to be one of the primary cause of antibiotic drug resistance in both humans and animals, livestock and poultry are play an important reservoirs of this bacteria (Diaz-Sanchez et al., 2015; Guetiya Wadoum et al., 2016; Yassin et al., 2017). The spread of multi-drug resistant (MDR) APEC strains is a critical and complex global health concern that could have a substantial impact on food safety and animal welfare. Because of antibiotic misuse in the chicken industry, the emergence and spread of *E. coli* strains that are resistant to antibiotics has become a global issue. The APEC strains that are resistant to antibiotics have been found to activate resistant genes in other virulent strains of *E. coli* , and these resistant genes can easily be transferred and transmitted between APEC strains and human ExPEC strains (Johar et al., 2021; Afayibo et al., 2022).

1.2 Problem statement

Colibacillosis, which is caused by the APEC, has a significant morbidity and mortality rate in chicken. Numerous virulence determinants are present in *E. coli* strains that cause infections. Numerous investigations have demonstrated that APEC may contribute to colibacillosis by making virulence factors encoded by various genes more harmful.

The ExPEC are more closely connected to phylogenetic groups B2 and D than nonpathogenic strains are to groups A and B1. Virulence determinants that are not present in strains from groups A and B1 are typically found in strains related to phylogenetic groups B2 and D. When it comes to APEC from isolates of septicemia and yolk sac infections, phylogenetic groups B1 and C were commonly known. Furthermore, a trade-off has been shown between virulence and resistance. Antimicrobial resistance was shown to be more prevalent in strains that did not belong to the phylogenetic group B2. Both the virulence factors and antimicrobial resistance genes are usually associated with mobile elements such as plasmids that assist in their spreading between bacteria and, thus, need to be closely controlled and monitored. Nevertheless, there is a shortage of the knowledge at level of relationship between antimicrobial resistance, virulence determinants and phylogenetic groups in poultry. To determine the incidence of MDR APEC strains found in poultry in Palestine, more research is needed.

1.3 Study hypothesis

The correlation between virulence factors, antibiotic resistance rates to APEC isolates recovered from poultry and phylogenetic groups is complex and needs clarification. In addition, to the relationship between the distribution rate of virulence factors in MDR APEC isolates and non-MDR APEC isolates. This study helps to identify the relationship which may play a vital role in disease progression and accurate therapeutic selection for poultry that suffer from colibacillosis.

1.4 Importance of the study

Antimicrobial resistance and molecular characterization (phylogenetic grouping and virulence factor detection) of APEC strains in North Palestine are the main topics of this investigation. The information gathered from this study will help us to understand the genetic diversity of APEC strains that were isolated from chicken, how to treat them, and how virulence factors cause colibacillosis to progress. This study will also provide information on the associations between phylogenetic groups, antibiotic resistance, and virulence factors.

1.5 Objectives

Regarding the APEC isolates found in Palestine, this study aimed to detect the phylogenetic groups, MDR and virulence-associated genes detected from APEC isolates recovered from the North of Palestine. Because of their various interconnections, the relationship between phylogenetic groups, antibiotic resistance, and APEC virulence factors is a complex process, this study also aimed to assess whether the virulence factors are directly associated with antibiotic resistance or, instead, dependent on a phylogenetic group distribution.

1.6 Literature review

Many studies reported the antimicrobial sensitivity among APEC isolates from healthy or non-healthy chicken. In one study, it was shown that all APEC isolates were MDR and possessed phenotypic resistance to more than 2 different antimicrobial agents. Resistance rate to the following tested antibiotics: ampicillin, tetracycline, ciprofloxacin and chloramphenicol was 98.6%, 97.3%, 72%, and 69.3% of isolates, respectively (Azam et al., 2019).

Meanwhile, they exhibited a high degree of resistance to trimethoprim, ampicillin and nalidixic acid (90.9%) as well as ciprofloxacin, chloramphenicol, norfloxacin and enrofloxacin (81.8%). Conversely, a moderate susceptibility of 63.6% was detected against colistin sulfate. All these clinical isolates showed MDR with at least 8 antibacterial agents (FR et al., 2019).

The APEC isolates recovered from poultry that diagnosed clinically with colibacillosis showed a resistance rate of 83.5%, 65.8%, 64.6%, 46.8% and 46.8% for ampicillin, nalidixic acid, tetracycline, cephalothin and ciprofloxacin, respectively. Nonetheless, according to Kim et al., (2020), the resistance rates to the cefepime (fourth-generation cephalosporins) and the cefotaxime and ceftazidime (third-generation cephalosporins) were, respectively,6.3%, 22.8%, 17.7%.

The antibiograms for APEC isolates revealed that resistant rate was 100%, 100%, 97.2%, 97.2%, 55.5%, 50.0%, 50%, 19.4%, 11.1%, 8.33% for ampicillin, tetracycline, chloramphenicol, erythromycin, enrofloxacin, norfloxacin, ciprofloxacin, streptomycin, colistin and gentamicin, respectively. Among these APEC isolates 16 antibiotic resistance patterns were detected. All the isolates were MDR (Ievy et al., 2020).

In a different investigation, APEC isolates were recovered from both healthy and unhealthy chicken. The isolates from the unhealthy birds showed 100%, 100%, 97.8%, 80.3%, and 84.4% resistance rates to ampicillin, cephalothin, ciprofloxacin, tetracycline, and fosfomycin, respectively. In addition, *E. coli* bacteria found in healthy chicken showed resistance rates of 82.4%, %94, 100%, 82.4%, and 76.5% to ampicillin, cephalothin, ciprofloxacin, tetracycline and fosfomycin, respectively (Johar et al., 2021). Both *E. coli* isolates from healthy and unhealthy chicken demonstrated moderate resistance to clavulanic acid/amoxicillin, with percentages of 41.5% and 17.6%, respectively. However, *E. coli* isolated from non-healthy chicken expressed reduced resistance to gentamicin, cefuroxime, ceftriaxone, and piperacillin/tazobactam, with rates of 10.4%, 4.4%, 4.4%, and 1.5%, respectively. Moreover, *E. coli* isolated from healthy chicken showed reduced resistance to gentamycin, with an 11.8% resistance rate (Johar et al., 2021).

A study conducted recently revealed that APEC isolates exhibited a high proportion of resistance to erythromycin, enrofloxacin, tetracycline, doxycycline, lincomycin, streptomycin, ampicillin, sulfamethoxazole, amoxicillin and cefalotin, was 98.7%, 96.1%, 95.2%, 93.9%, 90.0%, 90.0%, 87.8%, 84.3%, 81.7% and 78.7%, respectively. Isolates of APEC that showed moderate resistance rates to florfenicol, amikacin, gentamicin, lomefloxacin and cefotaxime, were 69.6%, 67.4%, 62.2%, 61.3% and 52.6%, respectively. However, APEC isolates showed very low resistance rate to polymyxin B and imipenem, was 0.4% and 1.7% , respectively. Moreover, all APEC isolates exhibit MDR (Afayibo et al., 2022).

On the other hand, the APEC isolates recovered from local chicken recorded a high resistance rate only to amoxicillin (78.6%) and tetracycline (76.2%). In addition, all APEC isolates recovered from commercial chicken were sensitive to colistin and all APEC isolates collected from native chicken were sensitive to gentamicin and meropenem. Furthermore, the most often detected resistance patterns were amoxicillin - enrofloxacin - nalidixic acid- sulfamethoxazole/trimethoprim - tetracycline (17.8%) for the APEC isolates recovered from commercial chicken and amoxicillin - tetracycline (59.5%) for the APEC isolates collected from local chicken isolates. The APEC isolates recovered from commercial broilers were 80% MDR to 3 or more antimicrobial classes,

while those isolated from local chicken were only 14.3% (Thomrongsuwannakij et al., 2022).

In another study, all APEC isolates that recovered from broiler breeders with colibacillosis showed low resistance rate 28.6%, 18%, 14.3%, 7.1%, 3.6% for Tetracycline, streptomycin, kanamycin, and gentamicin and sulfamethoxazole-trimethoprim, respectively. Unexpectedly, 10.7% of the isolates revealed resistance against more than 2 antibiotic classes. However, all isolates were sensitive to nalidixic acid, chloramphenicol, ciprofloxacin, ampicillin/ sulbactam, and cefotaxime (Joseph et al., 2023).

The prevalence of APEC isolates among phylogenetic groupings is demonstrated by numerous investigations. It was reported that APEC isolates recovered from commercial boilers belonged to phylogenetic groups D, B2, B1, and A with prevalence rates 41.1%, 31.1%, 22.2% and 5.6%, respectively. While those isolated from local chicken belonged to groups A, B2, B1 and D with a prevalence rate of 35.7%, 30.9%, 26.2% and 7.1%, respectively. Significant difference was observed ($P < 0.05$) between APEC strains recovered from commercial and local chicken that belonged to groups A and D (Thomrongsuwannakij et al., 2022).

In a different investigation, phylogenetic group B2 (71.4%) accounted for the most common of APEC isolates collected from broiler breeders suffering from colibacillosis. The phylogenetic groupings D and B1 showed lower prevalence, at 25% and 3.6%, respectively. Phylogenetic group A was not detected among these APEC isolates (Joseph et al., 2023).

According to a recent study, B1 (19.7%), E (18.7%), and A (13.6%) were the most common phylogroups among APEC recovered from distinct varieties of commercial chicken. There was a strong correlation between the broiler and the E group (26.4%), A group (15%), and B1 group (13.9%). Whereas similar association between layer breeders and broiler breeders in the B1 group (33.3%, 38.4%), B2 group (19%, 14.3%), and D group (9.5%,13.4%), respectively. However, in the event of the breeder 19.8% for the B2 group, 17.3% for the C group, and 16% for the group A, were higher. Interestingly, group D in the case of broiler, group F in the case of broiler breeder, and group C in the case of broiler breeder and layer breeder were entirely lacking from the APEC strains. Of the

isolates, 18.1%, (broiler (22%) and layer (17.3%)) had four genes classified as unknown (Bhattarai et al., 2023).

It was found that the APEC isolates had a higher prevalence rate of virulence-associated genes when compared to *E. coli* isolates collected from environmental and fecal samples. The 89.5%–94.7% of APEC strains had the virulence genes *iss*, *ompT*, *iroN*, *iutA* and *hlyF* identified while 10%–25% of environmental *E. coli* strains recovered from healthy chicken flocks and 46.6%–53.3% of avian fecal *E. coli* (AFEC) strains had these virulence factors. The bulk of APEC isolates were categorized in groups A and D, with only 3.1% and 11.7% of the total identified as belonging to phylogenetic groups B2 and B1, respectively (Hussein et al., 2013).

About 43% of the APEC isolates that were obtained from infected chicken exhibited at least six virulence genes, which indicates a high frequency of virulence determinants. The percentages of the most common virulence factors, *iss*, *iutA*, and *ColV*, were found to be 84%, 74.6%, and 60%, respectively. Approximately, 29% of APEC isolates had a combination of the virulence factors *iss*, *tsh*, *iroN*, and *iutA* (Azam et al., 2019).

In the APEC isolates recovered from chicken suffered from colibacillosis, the occurrence of virulence factors *iutA*, *hlyF*, *iroN*, *ompT*, and *iss* were 28.3%, 28.3%, 40%, 43.3%, and 38.3%, respectively. In addition, 43.3% of isolates harbored more than two virulence genes and 20% of isolates carried all 5 detected virulence genes. The gene profile *iutA*, *hlyF*, *iroN*, *ompT*, *iss*, which composed of 20% of isolates was predominant one. None of the detected virulence factors were identified in 56.6% of the total isolates (Haghighi Khoshkhoo et al., 2019).

The virulence genes reported from APEC isolates recovered clinically confirmed colibacillosis-affected chicken. The prevalence of the analyzed genes *hlyF*, *iutA*, *ompT*, *iroN* and *iss* was 93.7%, 91.9%, 89.9%, 79.9% and 78.5%, respectively. The distribution of phylogenetic groups was 46.8%, 7.6%, 22.8% and 22.8%, for D, B2, B1 and A, respectively. All phylogroup B2 isolates had all detected virulence genes. In addition, groups A/B1 and B2/D were significantly different according to the virulence gene *iss* ($P < 0.05$) (Kim et al., 2020).

The prevalence rate of virulence determinants in APEC strains was 97.22%, 58.33% and 33.33% for the following genes *fimC,* i*ucD* and *papC*, respectively. A combination of 3

virulence determinants (*fimC, iucD, papC*) was in 19.4% of isolates, different combinations of two virulence genes were positive in 50% of isolates and 30.6 were carrying one virulence gene. The most common combination (30.6%) of the two genes was *fimC* and *iucD* (Ievy et al., 2020).

The prevalence of different virulence determinants in pathogenic APEC isolates was 99%, 99%,97%,81%, 5%, 97%, 89%, and 81% for *ompT, hlyF*, *iroN*, *tsh*, *vat*, *iss*, *cvi/cva* and *iucD* gene, respectively. While, the prevalence of these determinants in non-pathogenic *E. coli* was 16%, 16%, 16%, 11%, 0%, 16%, 4%, 1% for *ompT, hlyF*, *iroN*, *tsh, vat, iss*, *cvi/cva* and *iucD* gene, respectively. Of the total APEC strains, 4.85% had all detected 8 virulence factors, 54.37% had 7 virulence factors, 29.12% had 6 virulence factors, and 10.68% had only 5 virulence factors (Johar et al., 2021). Alternatively, in non-healthy chicken the virulence determinants were evaluated at the following rates 69%, 69%, 68%, 54%, 4%, 70%, 59% and 65% for *ompT, hlyF*, *iroN*, *tsh*, *vat*, *iss*, *cvi/cva* and *iucD* gene, respectively. While, in healthy chicken, the virulence factors were recognized at the following rates 78%, 78%, 74%, 70%, 0%, 65%, 65% and 0% for *ompT, hlyF, iroN, tsh, vat, iss, cvi/cva* and *iucD* gene, respectively (Johar et al., 2021).

In a new study, the virulence genes of APEC collected from infected ducks and chicken showed the most typical clinical symptoms and signs of colibacillosis. The prevalence rate was 99.57%, 91.74%, 91.30%, 83.04% and 80.43% for virulence genes *ibeB, fimC, mat, ompA* and *iss*, respectively. The *fyuA, iroN, irp2,cva/cvi, iucD* and *tsh* genes were detected in 40–81% of the total recovered APEC isolates. However, other observed virulence genes were detected in low prevalence rates 20.87%, 18.26%, 13.91% and 13.48% for the virulence genes *Vat, neuC, papC* and *ibeA*, respectively. In addition, it was reported that all APEC strains harbored more than 2 virulence determinants (Afayibo et al., 2022). Also, it was found that all phylotypes showed a broad distribution of the virulence determinants *ibeB, tsh, irp2, iroN, iss, mat,* and *fimC.* These APEC isolates were phylogenetically grouped into B2, A, D, and B1 with a prevalence rate of 29.57%, 26.96%, 20.00% and 18.26%, respectively (Afayibo et al., 2022).

However, the previous study showed 5.22% of isolates were not allocated to any of the known groups. Certain groups were associated with certain virulence factors, as demonstrated by the finding that groups B1 and D had higher prevalence of *papC* and *vat* virulence factors than groups A and B2. The virulence determinants *fyuA, iucD,* and

cva/cvi were widely distributed with a prevalence rate of 84.78% for group D, 80.95% for group B1 and 80.65% for group A, respectively. Furthermore, the *vat* and *neuC* genes had a low prevalence rate in group A strains, although phylotype A strains were the main group with the gene *ibeA* (Afayibo et al., 2022).

In a recent study, during colibacillosis outbreaks in laying hens, the following virulence genes were identified in APEC isolates with prevalence rate; 90% for *iss*, 85% for *icuD*, 70% for *cvi/cva*, 70% for *irp2*, 63% for *tsh*, 35% for *vat*, 24% for *papC* and 6% for *astA* (Gambi et al., 2022).

Furthermore, it was found that one or more virulence genes were present in 96.4% of the APEC isolates that were collected from broiler breeders who had colibacillosis. The prevalent of the virulence genes was 78.6%, 78.6%, 78.6%, 78.6%, 68.0%, 42.9%,39.3%, 28.6%, 14.3% for virulence gene *iroN*, *ompT*, *hylF*, *iss*, *iutA*, *papC*, *tsh*, *cva/cvi* and *ibeA* , respectively (Joseph et al., 2023).

According to Bhattarai et al., (2023), the proportion of positive APEC isolates in commercial broilers and layers was high (91%). This study showed that The number of genes detected per APEC isolate varied from 8 to 26 out of the 57 virulence factors examined; the prevalence rate for the highest 5 virulence factors were 100% for *fimH*, 92.2% for *issa*, 90.6% for *traTa*, 86% for *sat chro*., and 84.8% for *ironEC*. There were notable variations in the gene frequency amongst the types of chicken (Bhattarai et al., 2023).

Chapter Two

Materials and Methods

2.1 Study design and isolate collection

2.1.1 Ethical approval and informed consent

In this type of study ethical approval is not required. However, during the collection of samples, the animals was handled carefully. Prior consent was taken from the owner of the animal farm.

2.1.2 Sample collection

A total of the 65 chicken clinically diagnosed with colibacillosis comprised a pool of samples. Each pool was composed of the samples of liver, heart, peritoneum and lung from fifty broilers' farms located in the northern region of Palestine. These samples were collected from dead chicken of various ages, having lesions of egg peritonitis, airsacculitis, pericarditis and perihepatitis. These samples were collected aseptically using a sterile cotton swab in a sterile 5 ml nutrient-rich broth Tryptone soy broth (TSB) in a lab belonging to these farms, during May to July 2024.

2.2 Phenotypic *E. coli* identification

Pre-enrichment samples in Tryptone soy broth (TSB) incubated for 6 h at 37 ◦C and then sub-cultured onto the Eosin Methylene Blue agar (EMB agar). To promote bacterial growth, the plates were incubated at 37^{o}C for 18-24 hours. Green metallic sheen colonies were selected for further confirmation by biochemical tests. Gram-staining method was used to investigate the morphological and staining properties of the bacteria and to provide knowledge regarding the possible bacterial identification. Isolated organisms with supporting growth characteristics were identified. A variety of biochemical tests, including Methyl Red (MR) Test, Catalase test, Voges-Proskauer (VP) test, Indole test, Simmons's citrate, Sulfide Indole Motility (SIM) medium test and Triple Sugar Iron (TSI) agar, were carried out to confirm identification of the specific *E. coli* bacteria (Habib et al., 2021). Further confirmation of the positive samples was done by specific PCR primers for the housekeeper alkaline phosphatase (*phoA*) gene.

2.3 Media preparation

2.3.1 Tryptone soy broth (TSB)

The instructions of the manufacturer which were labeled on the container were followed to prepare the tryptone soy broth. A 1L bottle was loaded with 500 ml deionized water and 15 g of TSB medium, mixed and heated until the TSB medium was completely dissolved. After that, the broth was then dispensed into tubes to have approximately 7-10 ml each, and plugged with a cotton. The tubes were autoclaved for 15 minutes at 121°C, after which they were left at room temperature to cool, then stored at 4°C in the refrigerator for one month.

2.3.2 Eosin Methylene Blue (EMB) agar

The instructions of the manufacturer, were followed to prepare the EMB medium. An 18.75 g of EMB Agar and 500 ml of deionized water were placed together into a 1L bottle, which was heated and shaken until the agar completely dissolved. After one minute of boiling, the solution was autoclaved for fifteen minutes at 121°C. The agar was then allowed to cool before 20 ml-35 ml was poured into sterile Petri plates, covered, and left overnight. The Petri dishes were flipped over and kept in the refrigerator the next morning.

2.3.3 Methyl red-Voges Proskauer (MR-VP)

About of 4.25 g of MR-VP medium and 250 ml of deionized water were mixed completely in a 0.5L bottle, and the mixture of medium was dissolve by heating. Approximately, 5-7 ml MR-VP broth was transferred into tubes, sterilized by autoclave at 121°C for 15 min, left at room temperature to cool, and then refrigerated at 4°C -6°C until use.

2.3.4 Simmons citrate agar

Simmons citrate agar was made in accordance with the directions provided by the manufacturer. Approximately 11.25 g powder of Simmons citrate agar and 500 ml of deionized water were mixed in a 1L bottle, and boiled until the agar completely dissolved. After that, 10 ml of Simmons citrate agar were placed in tubes, they were autoclaved for 15 minutes at 121°C. Slant agar tubes of this medium Simmons citrate agar were prepared, which then tubes were refrigerated at 4°C-6°C until use.

2.3.5 Triple sugar Iron agar

A 32.5g of triple sugar iron agar and 500 ml of deionized water were mixed effectively in a 1L bottle, and the mixture was heated to dissolve completely the agar. After the Triple Sugar Iron medium was distributed into tubes with about 10 ml, they were autoclaved for 15 minutes at 121°C. The slant agar tubes were prepared, which were subsequently refrigerated at 4°C-6°C until use.

2.3.6 Sulfide Indole Motility (SIM) medium

The manufacturer's directions, which are printed on the label of the container, were followed in preparing the SIM medium. Heat and stirring were used to dissolve the 7.5 g of SIM agar in a 0.5L bottle that contained 250 ml of deionized water. The SIM medium was poured into the tubes to a depth of approximately 5 cm. Following a 15-minute autoclave at 121 °C, the medium was allowed to cool before being kept in the refrigerator.

2.4. Identification of *E. coli* isolates

2.4.1 Eosin Methylene Blue (EMB) agar

Eosin methylene blue agar (EMB) is a selective and differential medium used to distinguish between lactose fermenters and non-fermenters bacteria. It contains two dyes—methylene blue and eosin, salts and lactose. The dyes inhibit Gram-positive bacteria from growing in EMB. Additionally, when bacteria such as Gram-negative use lactose as a sucrose of energy and carbon, the dyes react with the acidic chemicals and Gram-negative bacterial colonies, like *E. coli*, exhibit a green, metallic sheen when they generate a lot of acidic compounds (Willey et al., 2008)**.**

2.4.2 Gram staining

Gram-positive bacteria, which keep the crystal violet (primary dye), and Gram-negative bacteria, which have the color of the safranin O (counterstain), are distinguished by the Gram stain.

The variations in the cell wall structure cause these outcomes. Microorganisms are classified as Gram-negative or Gram-positive, due to the crystal violet is stabilized into the cell wall peptidoglycan layer by the next step, which involves the mordant Gram's iodine. Gram-negative bacteria have a thinner peptidoglycan layer than Gram-positive bacteria do. As a result, more crystal violet gets trapped in the cell wall of Gram-positive

bacteria. In the third phase, known as alcohol decolorization, lipids in outer membrane of Gram-negative bacteria dissolved and the crystal violet is removed from the peptidoglycan layer. Conversely, in Gram-positive microbes, the crystal violet is comparatively inaccessible and is not easily eliminated by alcohol. Only colorless Gram-negative bacteria are capable of accepting the counterstain, safranin, following the alcohol stage.

Gram staining was performed using fresh bacterial broth culture using the previously mentioned protocol (Green & Goldman, 2021). Gram-positive bacteria in this method have blue or purple color, while Gram-negative bacteria have red or pink color.

2.4.3 Indole test

The test for indole synthesis identifies bacteria that produce tryptophanase enzyme, an enzyme that can split tryptophan into indole. For an indole tube test, semisolid medium with a high tryptone content can be utilized. For 24–48 hours, a SIM agar deep tube was inoculated using the stab technique. Kovac's reagent, which contains 5% p-dimethylaminobenzaldehyde, was added in a few drops. A ring of pink color was produced at the surface of the SIM medium, indicating the organism produces tryptophanase and indole test positive. This technique may be used to screen for indole-positive *Enterobacteriaceae*, such as *E. coli*, and numerous others (Green & Goldman, 2021).

2.4.4 Methyl Red-Voges-Proskauer (MR-VP) test

The methyl red test is used to evaluate a microorganism's capacity to sustain a low pH over a lengthy (48–72 hours) incubation period in addition to producing strong acid from glucose. The MR-VP medium is inoculated and incubated for at least 48 hours. One mL aliquot of test broth is mixed with five drops of 0.02% MR reagent. A positive reaction is demonstrated by the medium becoming red.

The capacity of an organism to create acetylmethylcarbinol, or acetoin, from glucose metabolism is assessed using the VP test. After incubating the MR-VP broth for at least 48 hours, 0.6 mL of 5% alpha-naphthol and 0.2 mL of 40% potassium hydroxide are added to a 1 mL aliquot of test broth and gently mixed. If acetylmethylcarbinol is present, it is transformed to diacetyl by the 40% potassium hydroxide. The red coloration of diacetyl is caused by alpha-naphthol. The *Enterobacteriaceae* genera and species may

be distinguished from one another using the MR and VP tests. Certain *Enterobacter* species are negative for MR but positive for VP, whereas *Salmonella*, *Shigella* spp., *Citrobacter* and *E. coli* are negative for VP but positive for MR. Due to their both MR and VP positivity, a number of *Klebsiella* spp., and *Serratia* may be identified from other *Enterobacteriaceae*. At 25°C, *Yersinia enterocolitica* is VP test positive however, the test is variable at 35°C-37°C (Green & Goldman, 2021).

2.4.5 Citrate utilization test

One key feature in the identification of Enterobacteriaceae is the bacterium's capacity to use citrate as its restricted carbon source. In this medium, sodium citrate is considered the only supply of carbon, ammonium phosphate is considered the only source of nitrogen, and bromothymol blue dye is used as a pH indicator in the Simmons Citrate Medium formulation. The citrate usage test was conducted by the earlier description (Green & Goldman, 2021). Because the reaction needs oxygen, the cap of the tube was left loose. The agar slant was gently subcultured by stabbing and streaking inoculation. Then incubated for

up to four days at 35°C–37°C with the lid of the tube loosened. In case the organism used the citrate, ammonium phosphate was broken down to create ammonia and the growth occurred on the agar slant. As a result, the pH indicator changed from green to blue due to an alkaline pH shift. Many additional *Enterobacteriaceae* are citrate-positive, however, *Shigella* species and *E. coli* are not citrate-positive (Green & Goldman, 2021).

2.4.6 Triple sugar iron test

In bacteria, glucose fermentation can be evaluated via Triple sugar iron (TSI) agar analysis. This medium contains 3 types of sugars; lactose, glucose and sucrose. In addition, peptone and casein base with a pH-sensitive phenol red dye (pH indicator). Using an inoculum that has grown for 18 to 24 hours, slant agar is inoculated by stabbing method through the medium center and then streaking the surface of slant. Acid will be created in the agar if the organism ferments glucose, decreasing the pH and turning its red color into yellow. The medium may fracture or form cracks if gas is generated during fermentation process. The majority of *Enterobacteriaceae* species will exhibit an acid reaction, frequently accompanied by gas generation, during a 24-hour incubation period at 35°C–37°C. A mild acid reaction that may need to 48 hours to manifest and usually

involves no gas generation is produced by certain genera of Gram-negative rods. On TSI media, non-fermentative Gram-negative organisms such as *Moraxella* species and *Pseudomonas* species do not change color and have an alkaline pink reaction when they break down the peptones in the agar base.

In addition to sodium thiosulfate and iron salts, TSI agar is used to measure a Gram-negative organism's capacity to generate hydrogen sulfide (H_2S). If any H_2S is generated, it will combine with the iron salts in the TSI tube's butt to form a black precipitate. One of the most important tests for distinguishing *Enterobacteriaceae* species such as *Edwardsiella tarda*, *Proteus*, *Citrobacter*, and *Salmonella* from one another is the production of H_2S (Green & Goldman, 2021).

2.4.7 Catalase test

Catalase is an enzyme that breaks down Hydrogen peroxide to produce water and oxygen gas. After an organism grows for eighteen to twenty-four hours, drops of 3% hydrogen peroxide were applied. If the microorganism produces the catalase enzyme, oxygen bubbles are developed. Red blood cells contain catalase, which might lead to a false-positive result in catalase testing. Therefore, any medium containing whole blood should not be utilized. Chocolate agar may be used for the catalase test even if it includes blood since the erythrocytes were destroyed during the preparation of the media (Green & Goldman, 2021).

2.4.8 Motility

According to the procedure described by Green & Goldman, (2021), motility is shown macroscopically (naked eye) by making a straight stab (stabbing method) of growth into a semisolid medium, such as SIM, incubating it at 35°C–37°C for 24–48 hours , and then noticing whether the microorganism spreads out of the stab line, causing the neighbouring medium to become apparently turbid.

There are several motility medium compositions available. Tests for *Enterobacteriaceae* can be conducted using a tryptose-based 5% agar medium. The bacterial growth can be more easily seen if 2,3,5-triphenyl tetrazolium chloride (TTC) is added to this medium. The bacterial cells become reddish-colored due to TTC, which assists in clarifying the motility reaction. To examine non-fermentative bacteria that fail to grow on 5% agar medium, 3% agar containing yeast extract and casein peptone might be used. Fastidious

organisms may exhibit improved motility in a 1% agar medium supplemented with tryptose and heart infusion. *Klebsiella* and *Shigella* spp. are never motile (Green & Goldman, 2021).

2.5 Antibiotic resistance test

Using the Kirby Bauer test (disk diffusion method), antimicrobial resistance was identified in accordance with the Clinical and Laboratory Standard Institute (CLSI) instructions (CLSI., 2017). Based on their chemical structure of antimicrobial agents, all APEC isolates were evaluated for resistance to nine distinct classes: Aminopenicillin (Amoxicillin (30µg)), Cephalosporines (ceftriaxone (30µg), Cephalexin (30µg), Ceftiofur (30µg)), Tetracyclines (Doxycycline (10µg)), Fluoroquinolones (Ciprofloxacin (10µg), Norfloxacin (10µg), Enrofloxacin (5µg)), Aminoglycosides (Gentamicin (10µg), Neomycin (30µg)), Sulfonamides (Trimethoprim/Sulphamethoxazole (1.25 /23.75µg)), Polymyxins (Polymyxin B (10µg), Polymyxins E (Colistin (10µg)), Amphenicoles (Florfenicol (30µg)) and Phosphonic antibiotic (Fosfomycin (50µg)). A 6–8 hour-old culture of the APEC isolates was swabbed into Mueller Hinton agar (MHA) plates, then the antimicrobial disks were added to the APEC swabbed MHA plates. Following that, the plates were incubated at 37°C for 24 hours. The inhibition zones (if any) were determined according to the procedure of the CLSI (CLSI., 2017). The isolates were classified as sensitive, intermediate, or resistant. All antimicrobial susceptibility testing experiments involved the *E. coli* (ATCC 25922) strain as a quality control strain.

2.6 DNA extraction

Using the previously mentioned protocol, the genome of *APEC* isolates was extracted for PCR (Adwan et al., 2013). In short, a small number of colonies were removed from an overnight Mueller Hinton agar plate, mixed with approximately 1 milliliter of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then centrifuged at 14,000 X g for five minutes to pellet the cells. The pellet was re-suspended in about 0.5 milliliters of sterile distilled water and boiled for 10-15 minutes. After that, the samples were directly incubated on ice for 5-10 minutes. Centrifugation was used to remove the debris for five minutes at 14,000 X g. The extracted DNA samples were assessed for quality and quantity using a nanodrop spectrophotometer (GenovaNano, Jenway), then were kept at -20°C until they were needed for additional DNA analysis.

2.7 Molecular assays

2.7.1 Confirmation of *E. coli* isolates using PCR technique

To confirm *E. coli* isolates, the amplification housekeeping gene phoA was chosen as a particular target gene. The sequences of the primers and the size of the amplicon are given in Table 2.1. Each PCR reaction mixture had a total volume of 25 µl. A total of 12.5 µl of PCR premix containing MgCl2 (GoTaq® Green Master Mix, Promega), 0.4 µM of each primer, and three µl of DNA template were used for preparing the PCR reaction mixture. The DNA thermal cycler (Mastercycler personal, Eppendorf, Germany) was used to amplify DNA under the following conditions: 180 seconds at 94ºC followed by 30 cycles: 30 seconds at 94ºC, 30 seconds at 55ºC, and 30 seconds at 72ºC, with a final step 300 seconds at 72ºC. A volume of 20 µl of the product of the PCR reaction was subjected to electrophoresis using 1.5% agarose gel to identify the size of the amplified PCR band, after staining with 0.5 µg/ml of ethidium bromide dye. A 100-bp ladder (GeneDireX), was used in the electrophoresis.

2.7.2 Phylotyping of APEC isolates by PCR technique

By utilizing a triplex PCR, the APEC strains were categorized into one of the four phylogenetic groups (A, B1, B2, and D) according to the three DNA fragments chuA, yjaA, and TspE4C2 (Clermont et al., 2000). In this study, the sequences of the primer and their fragment sizes are presented in Table 2.1. According to earlier descriptions, the APEC isolates could be grouped into phylogenetic groups and sub-groups depending on the combination (presence or absence) of three PCR product fragments (Branger et al., 2005). As shown in Table 2.1. the APEC isolates were classified into phylogenetic groups and sub-groups based on the existence or lack of PCR product fragments. Each PCR reaction mixture had a total volume of 25 µl. For this PCR reaction mixture, 12.5 µl of PCR premix including MgCl2 (GoTaq® Green Master Mix, Promega), each primer 0.3 µM, and DNA template 3 µL (50-100 ng). Conditions for DNA amplification and electrophoresis as described in molecular identification of APEC isolates.

2.7.3 Virulence genotyping of APEC isolates by PCR technique

The extracted DNA was used to detect virulence genes. By used a total of 11 specific PCR primers, as listed in Table 2.2 for detection of virulence genes in *E.coli* isolated from chicken that have symptoms and signs of colibacillosis. The process of investigation used

multiplex PCR divided these genes into 3 pools. The genes that detected were *Iss*, *tsh*, *iutA*, *iroN*, *hlyF*, *ompT*, *papGI*, *papGII*, *papGI II*, papC and *ColV*. Primer sequences for these genes, size of amplicons, annealing temperatures and pools are listed in Table 2.2. Each 25 µL PCR reaction mixture was prepared as described in 2.7.2 Phylotyping of APEC isolates by PCR technique.

The DNA thermal cycler (Mastercycler personal, Eppendorf, Germany) was used to amplify DNA under the following conditions: one hundred eighty seconds at 94ºC, followed by twenty-five cycles: thirty seconds at 94ºC, thirty seconds at 58ºC, sixty seconds at 72ºC, and final step 300 hundred seconds at 72ºC. The PCR products were analyzed by electrophoresis to identify the size of the amplified PCR band, after staining with 0.5 µg/ml of ethidium bromide dye. A 100-bp ladder (GeneDireX), was used in the electrophoresis.

Table 2.1

Primer sequences were used in this study to confirm E. coli diagnosis and phylogenetic classification of E. coli isolates

Target genes	Primer sequence 5→3	Size pool (bp)	Reference
phoA	phoAF: TACAGGTGACTGCGGGCTTAT C phoA R: CTTACCGGGCAATACACTCACTA	622 0	(Liao et al., 2021)
yjaA	yjaA F TGAAGTGTCAGGAGACGCTG yjaA R ATGGAGAATGCGTTCCTCAAC	211 1	(Clermont et al., 2000)
ChuA	ChuA F GACGAACCAACGGTCAGGAT ChuA R TGCCGCCAGTACCAAAGACA	279 1	(Clermont et al., 2000)
TspE4C2	TspE4C2F GAGTAATGTCGGGGCATTCA TspE4C2R CGCGCCAACAAAGTATTACG	154 1	(Clermont et al., 2000)

Phylogroups and sub-groups result from applying the Clermont method

Gene PCR fragment*			Phylogroups and sub-groups assignment
ChuA	*yjaA*	*TspE4.C2*	
N	N	N	A (A0)
N	P	N	A (A1)
N	N	P	B1
N	P	N	B2 (B22)
N	P	P	B2 (B23)
P	N	N	D (D1)
P	N	P	D (D2)

*N: Negative; P: Positive

2.8 Statistical analysis

The antibiotic resistance scores (AR score) or virulence factor scores (VF score) for each isolate were calculated as the sum of all antibiotic resistance or virulence determinants that are found in each strain (J. R. Johnson et al., 2005). In this study, different statistical tests were carried out to analyze obtained data. These tests included Mann-Whitney U-Test (Two-tailed), T-Test Calculator for 2 Independent (Two-tailed) which used to test differences between the means of two groups, or the difference between one group's mean and Means Chi-square (χ^2) test which used to test the associations between groups. Values were considered statistically significant if $P < 0.05$.

Table 2.2

*Virulence gene primer sequences was used in this study, their amplicon sizes, annealing temperatures and pools**

Virulence Gene	Description	Primer sequence 5→3	Product size (bp)	Annealing temperature	Pool
iss	Increased serum survival gene (Protectins)	iss-F AGCAACCCGAACCACTTGATG iss-F AGCATTGCCAGAGCGGCAGAA	323	58	I
Tsh	Temperature-sensitive hemagglutinin gene (Adhesins)	tsh-F GGGAAATGACCTGAATGCTGG tsh-R CCGCTCATCAGTCAGTACCAC	420	58	I
iutA	Ferric aerobactin receptor gene; iron transport (Iron acquisition)	iutA-F GGCTGGACATCATGGGAACTGG iutA-F CGTCGGGAACGGGTAGAATCG	302	58	I
iroN	Catecholate siderophore receptor gene (Iron acquisition)	iroN-F AAGTCAAAGCAGGGGTTGCCCG iroN-R GACGCCGACATTAAGACGCAG	667	58	I
hlyF	Hemolysin F gene (Toxins)	hlyF-F GGCGATTTAGGCATTCCGATACTC hlyF-R ACGGGGTCGCTAGTTAAGGAG	599	58	I
ompT	Outer membrane protease gene (Protectins)	ompT-F ATCTAGCCGAAGAAGGAGGC ompT-R CCCGGGTCATAGTGTTCATC	559	58	I
papGI	Pyelonephritis-associated pili allele I (Adhesins)	papGI-F TCGTGCTCAGGTCCGGAATTT papGI-R TGGCATCCCCCAACATTATCG	461	58	II
papGII	Pyelonephritis-associated pili allele II (Adhesins)	papGII-F GGGATGAGCGGGCCTTTGAT papGII-R CGGGCCCCCAAGTAACTCG	190	58	II
papGIII	Pyelonephritis-associated pili allele III (Adhesins)	papGIII-F GGCCTGCAATGGATTTACCTGG papGIII-R CCACCAAATGACCATGCCAGAC	258	58	II
papC	Encode for P pilus (Adhesins)	papC-F TGATATCACGCAGTCAGTAGC papC-R CCGGCCATATTCACATAAC	501	58	III
cva/cvi	Colicin V operon	ColV-F TCCAAGCGGACCCCTTATAG ColV-R CGCAGCATAGTTCCATGCT	598	58	III

*(Azam et al., 2019)

Chapter Three

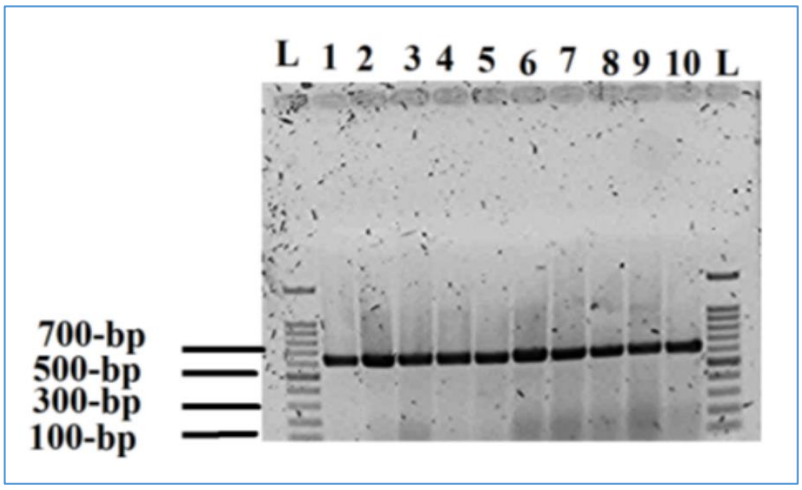
Results

3.1 Phenotypic identification of APEC isolates

The colonies of the isolated strains grown on EMB medium have a green metallic sheen, Gram stain negative, short rod, single, pair or in a short chain. All isolates were identified as lactose fermenter with gas production, Voges-Proskauer test negative, Methyl Red positive, motile test positive, citrate utilization negative, Indole test positive and H_2S production negative. Besides these tests, the PCR products of *E. coli*-specific *phoA* gene of 65 bacterial strains showed by gel electrophoresis an amplified fragment of the 622-bp band, this confirmed the identification of these *E. coli* isolates by PCR technique. Data is presented in Figure 3.1.

Figure 3.1

The PCR product of E. coli-specific phoA gene. Lanes L: 100-bp ladder, lanes 1-10 the PCR product (622-bp) band of E. coli-specific phoA gene



3.2 Isolation rate of APEC

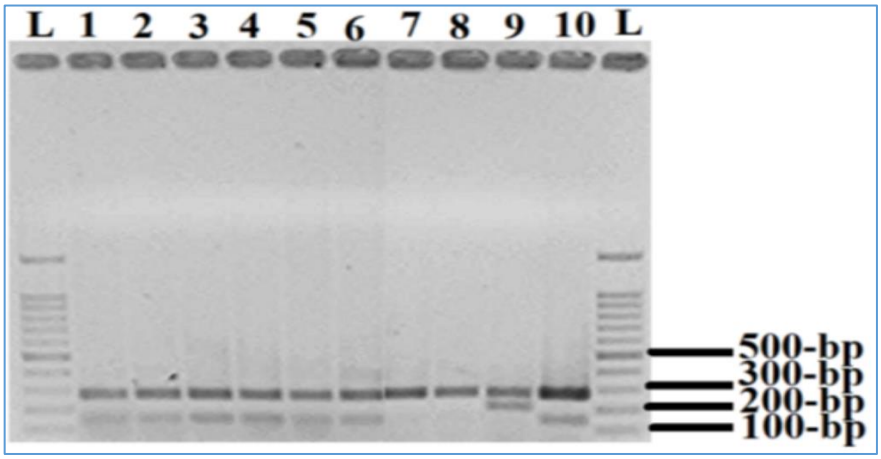
Among a total of the 65 chicken clinically diagnosed with colibacillosis, 65 APEC isolates were recovered 65 pools from four different infected sites in the chicken.

3.3 Phylotyping of APEC isolates by PCR technique

Triplex PCR analysis for phylogenetic classification showed that the most APEC strains 47/65 (72.3%) were belonged to the subgroup D2. Other strains were assigned to subgroup D1 (9/65, 13.8%), B2 (5/65, 7.7%), B1 (3/65, 4.6%) and A (1/65, 1.5%). Data is presented in Table 3.1 in Appendix and Figure 3.2. Statistical analyses showed that there was a significant difference between the prevalence of D group and other groups at P <0.00001 using Mann-Whitney U-Test, indicating that the genetic markers (*chuA*, *yjaA* and *TspE4.C2*) are differently distributed between the strains of D group and strains of other groups.

Figure 3.2

Phylogenetic groups for APEC isolates recovered from broilers' farms in northern Palestine using Triplex PCR.



Notes: Phylogenetic group D2 included lanes 1-6 and 10; group D1 included lanes 7 and 8; group B2 included lane 9; and lanes L comprised 100-bp ladder

3.4 Susceptibility of APEC isolates to antibiotics

The chemical structures of all tested antibiotics were categorized into nine distinct classes: Aminopenicillin (Amoxicillin), Cephalosporines (ceftriaxone, Cephalexin, Ceftiofur), Tetracyclines (Doxycycline), Fluoroquinolones (Ciprofloxacin, Norfloxacin, Enrofloxacin), Aminoglycosides (Gentamicin, Neomycin), Sulfonamides (Trimethoprim/Sulphamethoxazole), Polymyxins (Polymyxins B, PolymyxinsE (Colistin)), Amphenicoles (Florfenicol) and Phosphonic antibiotic (Fosfomycin). Antibiotic resistance ranges from 27.7% for PolymyxinsE (Colistin) to 100% for Amoxicillin. Data is presented in Table 3.2 and Figure 3.3. The 65 APEC isolates revealed fifty-eight resistance patterns, the most prevalent of which was N, FO, PB, FFC, CL, CN, FUR, AX, NOR, CIP, ENR, CRO, and STX. This suggests that the APEC strains

in these broiler farms in northern Palestine exhibit a wide variety of resistance patterns (Table 3.3 in Appendix). Results showed that 7 clusters depend on resistance/sensitive of 65 APEC strains to the antibiotics used in this study, but clustering process using dendrogram is independent to phylogenetic groups. In addition, Clusters C1, C2, C3 and C4 can be divided into 2 or 3 sub-clusters. Data is shown on Figure 3.4. This number of clusters and sub-clusters suggests that the APEC strains in these broiler farms in northern Palestine exhibit a wide variety of resistance patterns.

Polymyxins E (Colistin) and Fosfomycin were the most effective drugs against these APEC isolates. The isolates from group B1 were less drug-resistant than those from groups D and B2. An assessment was conducted on the association between the phylogenetic group and the mean antibiotic resistance score. The mean antibiotic resistance score was 11, 11.8 and 8.7 for groups D, B2 and B1 strains, respectively. A two-tailed t-test only showed that there was a significant difference between the aggregate antibiotic resistance scores between isolates resistant to norfloxacin phylogenetic groups D and B1. Also, there was a significant difference between the aggregate antibiotic resistance scores between isolates resistant to Ciprofloxacin, Norfloxacin and Cephalexin phylogenetic groups B1 and B2 ($P < 0.05$). Data is shown in Table 3.4. According to the prevalence of antibiotic resistance based on the phylogenetic groups, results showed a significant difference between the prevalence of Cephalexin resistance between groups B1 and B2 ($P < 0.05$). Data is shown in Table 3.4.

Table 3.2

The antibiotic resistance profile of 65 APEC isolates collected from broiler farms in northern Palestine.

Group	Antibiotic	Resistant strains No. of samples (%)	Intermediate strains No. of samples (%)	Susceptible strains No. of samples (%)
Tetracyclin	Doxycycline	32 (49.2)	11 (16.9)	22 (33.8)
Fluoroquinolone	Ciprofloxacin	56 (86.2)	8 (12.3)	1 (1.5)
	Norfloxacin	58 (89.2)	6 (9.2)	1 (1.5)
	Enrofloxacin	64 (98.5)	1 (1.5)	0 (0.0
Aminoglycoside	Gentamicin	52 (80.0)	5 (7.7)	8 (12.3)
	Neomycin	40 (61.5)	4 (6.2)	21 (32.3)
Sulfonamides	Sulfamethoxazole/trimethoprim	51 (78.5)	1 (1.5)	13 (20.0
Cephalosporins	Cephalexin	47 (72.3)	1 (1.5)	17 (26.2)
	Ceftriaxone	58 (89.2)	4 (6.2)	3 (4.6)
	Ceftiofur	60 (92.3)	5 (7.7)	0 (0.0)
Aminopenicillin	Amoxicillin	65 (100)	0 (0.0)	0 (0.0)
Polymyxins	Polymyxins E (Colistin)	18 (27.7)	2 (3.0)	45 (69.2)
	Polymyxin B	36 (55.4)	8 (12.3)	21 (32.3)
Amphenicoles	Florfenicol	55 (84.6)	1 (1.5)	9 (13.8)
Phosphonic Antibiotic	Fosfomycin	23 (35.4)	0 (0)	42 (64.6)

Figure 3.3

The antibiotic resistance profile of 65 APEC isolates collected from broiler farms in northern Palestine

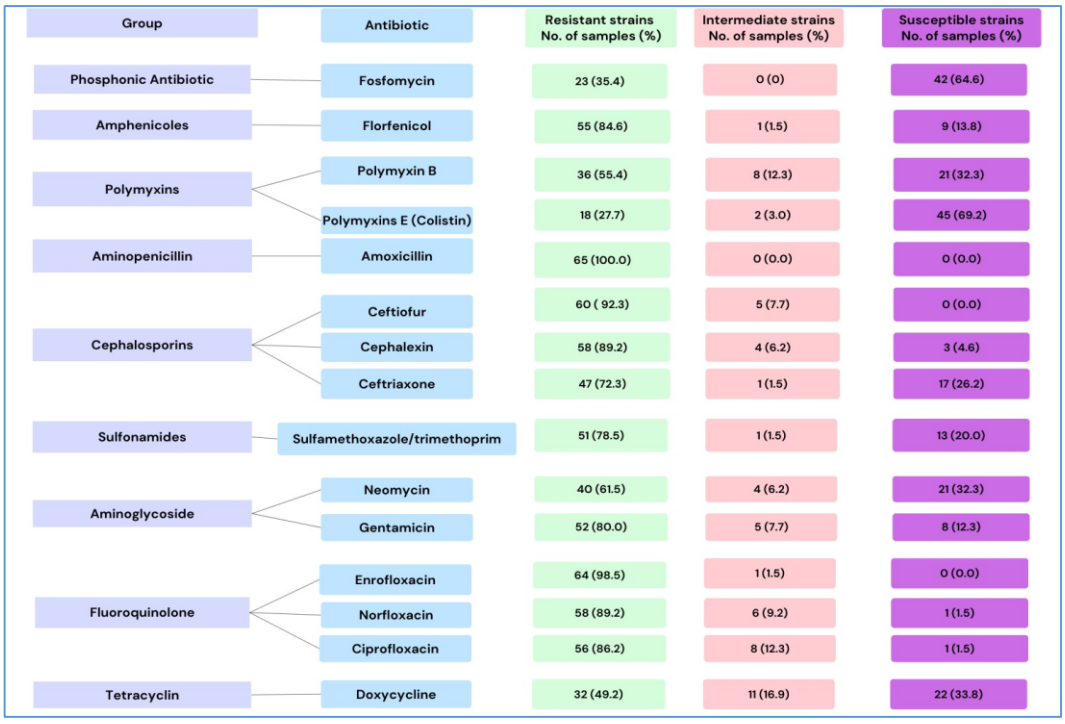
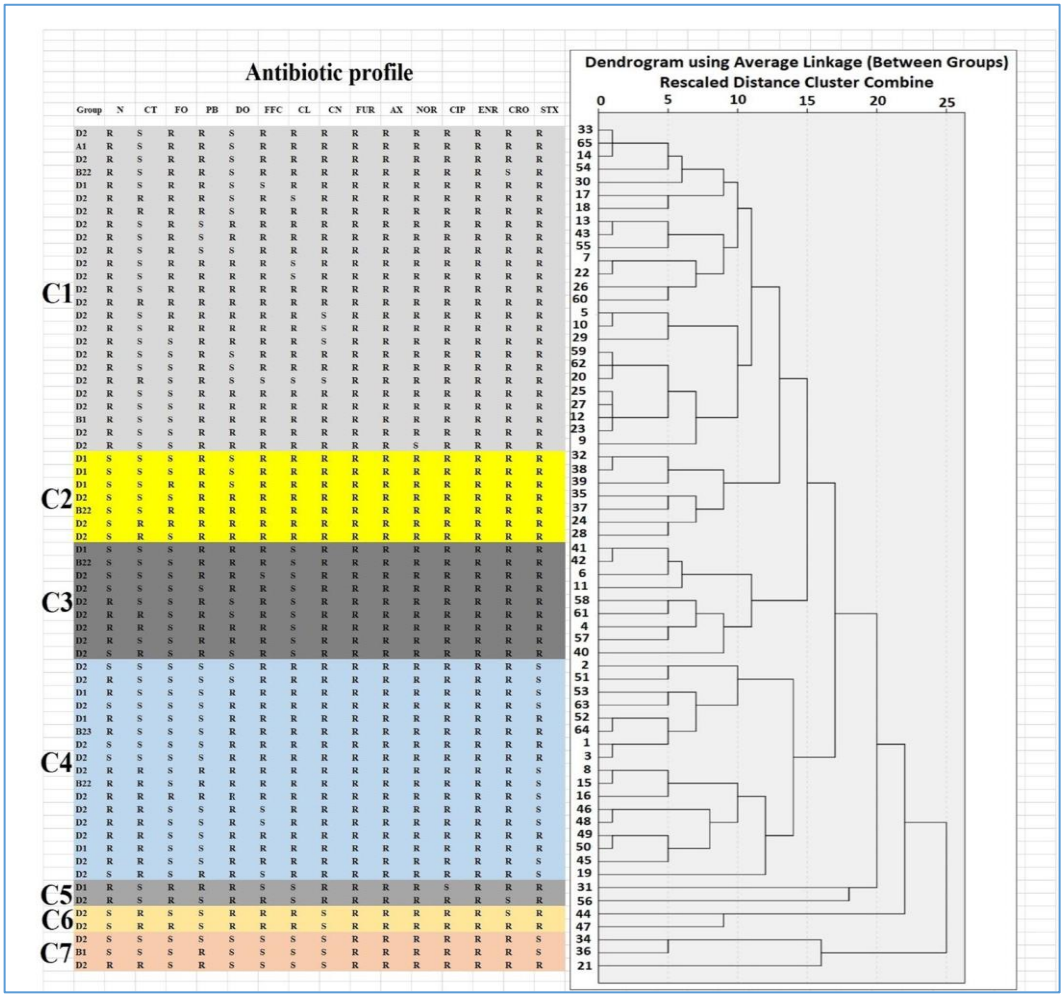


Figure 3.4

Dendrogram of 65 APEC strains isolated collected from broiler farms in northern Palestine



Notes: based on the UPGMA method derived from analysis of the antibiotic resistance profile and Phylogenetic groups. Intermediate antibiotic effect on APEC isolates considered resistant effect

N: Neomycin sulfate; CT: Colistin sulphate; PB: Polymyxin B; DO: Doxycycline; FFC: Florfenicol; CL: Cephalexin; CN: Gentamicin; FUR: Ceftiofur; AX: Amoxicillin; NOR: Norfloxacin; ENR: Enrofloxacin, CIP: Ciprofloxacin; CRO: Ceftriaxone; STX: Sulfamethoxazole/Trimethoprim; FO: Fosfomycin.

C: Cluster

Table 3.4

The antibiotic resistance of 65 APEC isolates collected from broiler farms in northern Palestine

Group	Antibiotic	Resistant Strains N (%)	Prevalence of AR between groups N (%)			Aggregate AR score (mean)		
			D	B1	B2	D	B1	B2
		N=65 (%)	N=56(%)	N= 3 (%)	N= 5 (%)	AR score (mean) 618 (11.0)	AR score (mean) 26 (8.7)	AR score (mean) 59 (11.8)
Tetracyclin	Doxycycline	32(49.2)	30 (53.6%)	1(33.3%)	1(20%)	342(6.1)	11(3.7)	13(2.6)
Fluoroquinolone	Ciprofloxacin	56 (86.2)	48 (85.7%)	2(66.7%)	5(100%)	542 (9.7)	16(5.3) [a]	59 (11.8) [a]
	Norfloxacin	58 (89.2)	50 (89.3%)	2(66.7%)	5(100%)	564 (10.1) [b]	16(5.3) [b, c]	59 (11.8) [c]
	Enrofloxacin	64 (98.5)	55 (98.2%)	3(100%)	5(100%)	613(10.9)	26(8.7)	59(11.8)
Aminoglycoside	Gentamicin	52 (80.0)	44 (78.6%)	2(66.7%)	5(100%)	504 (9)	21(7)	59 (11.8)
	Neomycin	40 (61.5)	34 (60.7%)	1(33.3%)	4(80%)	399 (7.1)	10(3.3)	46(9.2)
Sulfonamides	Sulfamethoxazole/trimethoprim	51 (78.5)	44 (78.6%)	2(66.7%)	4(80%)	498(8.9)	21(7)	47(9.4)
Cephalosporins	Cephalexin	47 (72.3)	40 (71.4%)	1(33.3%) [e]	5(100%) [e]	542(9.7)	10(3.3) [d]	59 (11.8) [d]
	Ceftriaxone	58 (89.2)	51 (91.1.0%)	3(100%)	4(80%)	571(10.2)	26(8.7)	47(9.4)
	Ceftiofur	60 (92.3)	52 (92.9%)	2(66.7%)	5(100%)	585(10.4)	21(7)	59 (11.8)
Aminopenicillin	Amoxicillin	65 (100.0)	56 (100.0%)	3(100%)	5(100%)	618(11)	26(8.7)	59(11.8)
Polymyxins	Polymyxins E(Colistin)	18 (27.7)	17 (30.4%)	0(0%)	1(20%)	200(3.6)	0(0)	12(2.4)
	Polymyxin B	36 (55.4)	30 (53.6%)	2(66.7%)	3(60%)	356 (6.4)	21(7)	37(7.4)
Amphenicoles	Florfenicol	55(84.6)	47 (83.9%)	2(66.7%)	5(100%)	537(9.6)	21(7)	59 (11.8)
Phosphonic Antibiotic	Fosfomycin	23 (35.4)	20 (35.7%)	0(0%)	2(40%)	250 (4.5)	0(0)	25(5)

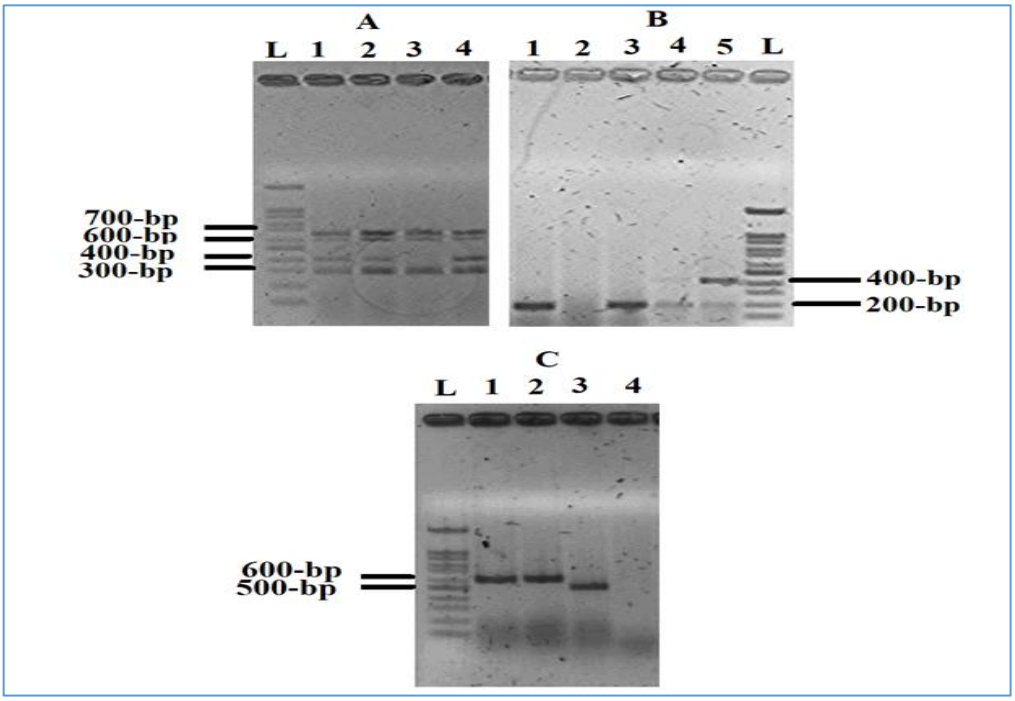
AR: Antibiotic Resistance; The aggregate AR score (mean) and prevalence of AR between groups did not include the isolate belonged the group A.

[a, b, c, d, e] Significant at $P < 0.05$ (t-test)

It was found that the most common virulence gene was iroN which was detected in 61 isolates (93.8%). While, 56 (86.2%), 42 (64.6%), 40 (61.5%), 37 (56.9%), 24 (36.9%), 23 (35.4%),16 (24.6%), 13 (20.0%), 0 (0.0%) and 0 (0.0%) isolates were positive for *hlyF, iutA, Tsh, ColV, papGII, Iss, papGI, papC, papGIII* and *ompT* genes, respectively. Data is shown in Figure 3.5 and Table 3.5. It was found that 36 (55.4%) isolates had at least 5 genes. Thirty-four virulence gene patterns were observed in the 65 APEC isolates with *Tsh, iutA, iroN,* and *hlyF* being the most predominant (12.3%). (Table 3.6.) Results showed that 6 clusters depend on the presence/absence of virulence factors in 65 APEC strains used in this study, but the clustering process using dendrogram is independent of phylogenetic groups. In addition, each of these clusters can be divided into 2 or more sub-clusters. (Figure 3.6.) This number of clusters and sub-clusters suggests that the APEC strains in these broiler farms in northern Palestine exhibit a wide genetic variation.

Figure 3.5

Multiplex PCR profiles specific for APEC virulence factors



Note: Lanes L have a 100-bp ladder. A: Genes utaA (302-bp), tsh (420-bp), hlyF (599-bp) and iroN (667-bp) are shown in Lanes 1, 2, 3 and 4. B: Genes papGII (190-bp) and papGI (461-bp) are shown in lanes 1, 3 and 5. C: Genes papC (501-bp) and cva/cvi (598-bp) are shown in lanes 1, 2 and 3.

A two-tailed t-test showed no significant difference between the aggregate virulence factor scores and phylogenetic groups D, B1 and B2 ($P < 0.05$). According to the distribution of virulence factors based on the phylogenetic groups, results showed a significant difference between the distribution of *cva/cvi* between group B1 and B2 ($P < 0.05$). Data is shown in Table 3.5.

It was also found that *iroN* and *hlyF* gene was the most common prevalence among strains resistant to fluoroquinolones, Cephalosporins, Aminoglycoside, Polymyxins, Fosfomycin, Doxycycline, Florfenicol and Trimethoprim/Sulphamethoxazole. The *iroN* gene prevalence was 95.2%, 95.3%, 94.6%, 93.2%, 100%, 96.9%, 94.4% and 96% for isolates resistant to fluoroquinolones, Cephalosporins, Aminoglycoside, Polymyxins, Fosfomycin, Doxycycline, Florfenicol and Trimethoprim/Sulphamethoxazole, respectively. The *hlyF* gene prevalence was 88.9%, 87.5%, 85.7%, 81.8%, 90.9%, 87.5%, 90.7% and 92% for isolates resistant to fluoroquinolones, Cephalosporins, Aminoglycoside, Polymyxins, Fosfomycin, Doxycycline, Florfenicol and Trimethoprim/Sulphamethoxazole, respectively. The *iutA* gene was the most common prevalence among strains resistant to Polymyxins (Tables 3.7-3.10).

In this study, significant associations ($P < 0.05$) between specific Virulence factor content and phenotypic resistance were detected among clinical APEC isolates. These include *iutA* and *iss* Polymyxins resistance (Table 3.8).

In addition, a two-tailed t-test showed that *papGII*, *Tsh*, *iutA*, *iss* virulence factors were more prevalent in isolates that showed polymyxins resistant phenotype than sensitive isolates, as well as more common in polymyxins resistant phenotype isolates that belonged to group D than sensitive isolates in the same group ($P < 0.05$) (Table 3.8.). The same *papGII* and *papC* virulence factors were more prevalent in isolates that showed Fosfomycin resistant phenotype than sensitive isolates. Also, the *papC* virulence factor is more common in Fosfomycin-resistant phenotype isolates that belonged to group D than sensitive isolates in the same group ($P < 0.05$) (Table 3.9.).

In addition, the *hlyF* virulence factor is more common in Florfenicol- and Sulfamethoxazole/trimethoprim- resistant phenotype isolates that belonged to group D than sensitive isolates in the same group ($P < 0.05$). However, *hlyF* virulence factor is more common in isolates that showed Sulfamethoxazole/trimethoprim-resistant phenotype regardless of the type phylogenetic group ($P < 0.05$) (Table 3.10.)

Table 3.5

Virulence genes, virulence factor scores, the prevalence of virulence factors and their distribution to the phylogenetic groups D, B1 and B2

Virulence genes		Prevalence	distribution of VF according to the phylogenetic groups			Aggregate VF score (mean)		
		N (%)	D N=56(%)	B1 N= 3(%)	B2 N=5 (%)	D N=56 272 (4.86)	B1 N=3 14 (4.67)	B2 N=5 25 (5)
Adhesins genes	*papGI*	16 (24.6)	12 (21.4)	2 (66.7)	2 (40.0)	65 (1.1)	9 (3.0)	12 (2.4)
	papGII	24 (36.9)	24 (42.9)	0 (0.0)	0 (0.0)	140 (2.5)	0 (0.0)	0 (0.0)
	papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	papC	13 (20.0)	13 (23.2)	0 (0.0)	0 (0.0)	77 (1.4)	0 (0.0)	0 (0.0)
	Tsh	40 (61.5)	34 (60.7)	2 (66.7)	4 (80.0)	181 (3.2)	10 (3.3)	22 (4.4)
Iron acquisition systems genes	*iutA*	42 (64.6)	36 (64.3)	3 (100)	3 (60.0)	176 (3.1)	14 (4.7)	15 (3.0)
	iroN	61 (93.8)	53 (94.6)	3 (100)	5 (100)	261 (4.7)	14 (4.7)	25 (5.0)
Cell Protectins genes	*Iss*	23 (35.4)	20 (35.7)	0 (0.0)	2 (40.0)	96 (1.7)	0 (0.0)	10 (2.0)
	ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)
Toxins genes	*hlyF*	56 (86.2)	49 (87.5)	3 (100)	4 (80.0)	242 (4.3)	14 (4.7)	22 (4.4)
Colicin V operon	*cva/cvi*	37 (56.9)	31 (55.4)	1(33.3) *	5 (100) *	160 (2.9)	5(1.7)	25 (5.0)

VF: Virulence factor; N: Number of isolates.

* significant at $p < 0.05$

One isolate belonging to group A is not included in the VF and VF score (mean) distribution.

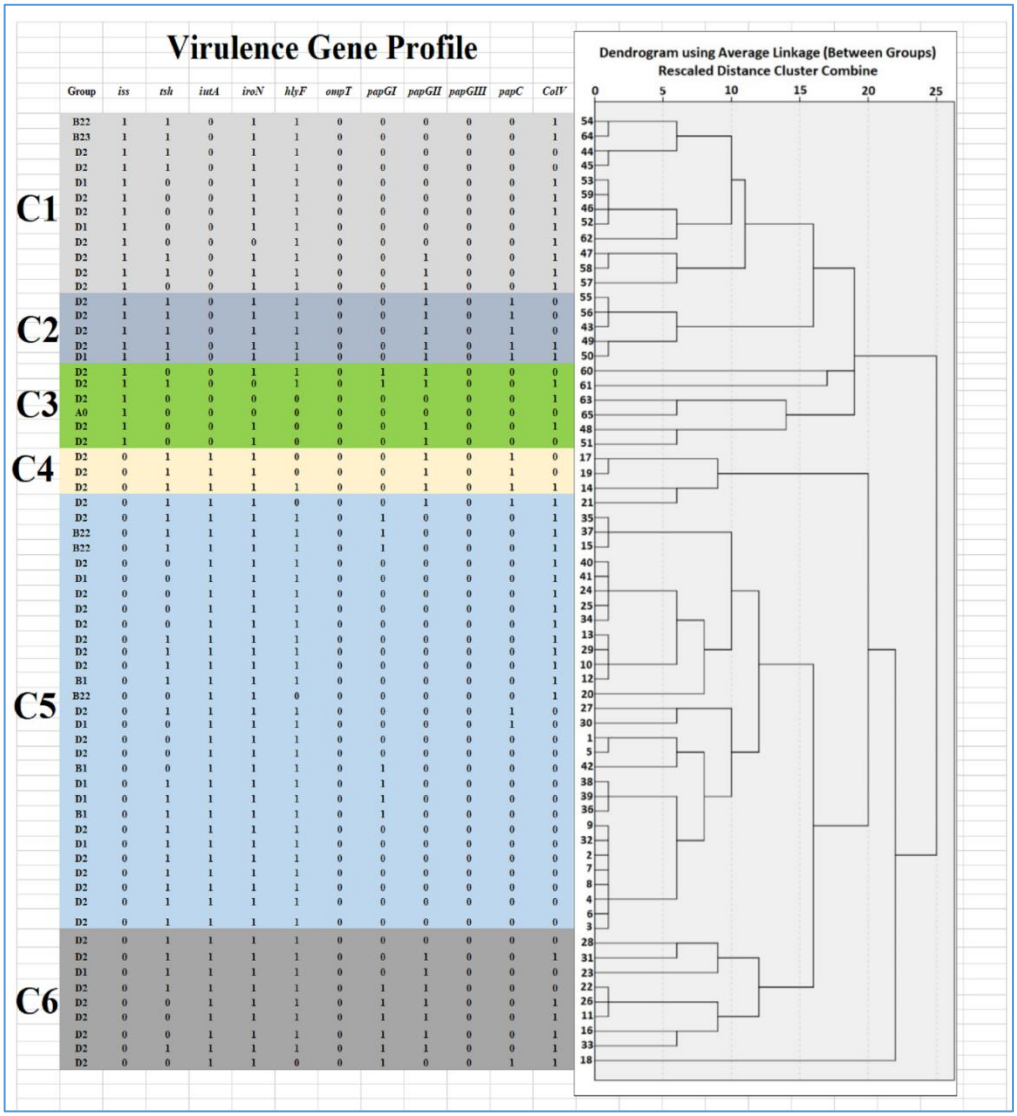
Table 3.6

Virulence gene patterns of 65 APEC isolates recovered from broilers' farms in northern Palestine

No.	Virulence gene patterns	No. of virulence gene patterns
1	*iutA, iroN, hlyF*	2
2	*Tsh, iutA, iroN, hlyF*	8
3	*Tsh, iutA, iroN, hlyF, cva/cvi*	4
4	*iutA, iroN, hlyF, papGI, papGII, cva/cvi*	3
5	*Tsh, iutA ,iroN, hlyF, papGII, papC , cva/cvi*	1
6	*Tsh, iutA ,iroN, hlyF, papGI, cva/cvi*	3
7	*Tsh, iutA ,iroN, hlyF, papGI, papGII, cva/cvi*	1
8	*Tsh, iutA ,iroN, papGII, papC*	2
9	*iutA, iroN,papGI, papC, cva/cvi*	1
10	*iutA, iroN, cva/cvi*	1
11	*Tsh, iutA ,iroN, papGII, papC , cva/cvi*	1
12	*Tsh ,iutA, iroN, hlyF, papGI, papGII*	1
13	*iutA, iroN, hlyF, cva/cvi*	5
14	*Tsh, iutA, iroN, hlyF, papC*	1
15	*Tsh, iutA ,iroN, hlyF, papGII, cva/cvi*	1
16	*iutA ,iroN, hlyF, papC*	1
17	*Tsh ,iutA, iroN, hlyF, papGII*	1
18	*Tsh ,iutA, iroN, hlyF, papGI, papGII, papC, cva/cvi*	1
19	*Tsh ,iutA, iroN, hlyF, papGI*	3
20	*iutA ,iroN, hlyF, papGI, papC*	1
21	*Iss, Tsh , iroN, hlyF, papGI, papC*	3
22	*Iss, Tsh , iroN, hlyF*	2
23	*Iss, iroN, hlyF, cva/cvi*	4
24	*Iss, Tsh ,iroN, hlyF, papGII, cva/cvi*	2
25	*Iss,iroN, papGII, cva/cvi*	1
26	*Iss, Tsh ,iroN, hlyF, papGII, papC, cva/cvi*	2
27	*Iss, iroN, papGII*	1
28	*Iss, tsh , iroN, hlyF, cva/cvi*	2
29	*Iss, iroN, hlyF, papGII, cva/cvi*	1
30	*Iss, iroN, hlyF, papGI, papGII*	1
31	*Iss, tsh , hlyF, papGI ,papGII, cva/cvi*	1
32	*Iss, hlyF, cva/cvi*	1
33	*Iss, cva/cvi*	1
34	*Iss*	1
Total		65

Figure 3.6

Dendrogram of 65 APEC strains isolated collected from broiler farms in northern Palestine



Note: based on the UPGMA method derived from analysis of the virulence factor profile and Phylogenetic groups

Table 3.7

Virulence factors in relation to Fluoroquinolones and Cephalosporins resistant phenotype among 64 APEC isolates recovered from broilers' farms in northern Palestine with colibacillosis

VF	No. (%) of APEC isolates							
	Fluoroquinolones resistant and susceptible phenotype							
	Group D n= (%)		Group B1 n= (%)		Group B2 n= (%)		Group D/B1/B2 n =(%)	Group D/B1/B2 n =(%)
	R (n= 55)	S (n=1)	R (n=3)	S (n=0)	R (n=5)	S (n= 0)	R (n=63)	S (n=1)
papGI	12 (21.8)	0 (0.0)	2(66.7)	0(0.0)	2 (40)	0 (0.0)	16(25.4)	0 (0.0)
papGII	23 (41.8)	1(100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	23 (36.5)	1 (100)
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papC	12 (21.8)	1(100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (19)	1(100)
Tsh	34(61.8)	0 (0.0)	2 (66.7)	0 (0.0)	4 (80)	0 (0.0)	40 (63.5)	0 (0.0)
iutA	35 (63.6)	1(100)	3 (100)	0 (0.0)	3 (60)	0 (0.0)	41(65.1)	1(100)
iroN	52 (94.5)	1(100)	3(100)	0 (0.0)	5 (100)	0 (0.0)	60 (95.2)	1(100)
Iss	20 (36.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40)	0 (0.0)	22 (34.9)	0 (0.0)
ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	49(89.1)	0(0.0)	3 (100)	0 (0.0)	4 (80)	0 (0.0)	56 (88.9)	0 (0.0)
cva/cvi	30 (54.5)	1(100)	1(33.3)	0 (0.0)	5 (100)	0 (0.0)	36 (57)	1(100)
	Cephalosporins resistant and susceptible phenotype							
R (n= 56)	S (n=0)	R n=3)	S (n=0)	R n=5)	S (n= 0)	R (n=64)	S (n=0)	
papGI	12 (21.4)	0 (0.0)	2(66.7)	0 (0.0)	2 (40)	0 (0.0)	16 (25)	0(0.0)
papGII	24 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (37.5)	0 (0.0)
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)
papC	13 (23.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (20.3)	0 (0.0)
Tsh	34(60.7)	0 (0.0)	2(66.7)	0 (0.0)	4 (80)	0 (0.0)	40 (62.5)	0 (0.0)
iutA	36 (64.3)	0 (0.0)	3 (100)	0 (0.0)	3 (60)	0 (0.0)	42 (65.6)	0 (0.0)
iroN	53 (94.6)	0 (0.0)	3(100)	0 (0.0)	5 (100)	0 (0.0)	61 (95.3)	0 (0.0)
Iss	20 (35.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (40)	0 (0.0)	22 (34.4)	0 (0.0)
ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	49(87.5)	0 (0.0)	3 (100)	0 (0.0)	4 (80)	0 (0.0)	56 (87.5)	0 (0.0)
cva/cvi	31 (55.4)	0 (0.0)	1(33.3)	0 (0.0)	5 (100)	0 (0.0)	37 (57.8)	0 (0.0)

VF: Virulence factor, R: Resistant; S: Susceptible.

Table 3.8

Virulence factors in relation to Aminoglycoside and Polymyxins resistant phenotypes among 64 APEC isolates recovered from broilers' farms in northern Palestine with colibacillosis

VF	No. (%) of APEC isolates Aminoglycoside-resistant and Susceptible phenotype							
	Group D n= (%)		Group B1 n= (%)		Group B2 n= (%)		Group D/B1/B2 n =(%)	Group D/B1/B2 n =(%)
	R (n= 49)	S (n=7)	R (n=2)	S (n=1)	R (n=5)	S (n= 0)	R (n=56)	S (n=8)
papGI	10 (20.4)	2 (28.6)	1 (50)	1(100)	2 (40)	0 (0.0)	13 (23.2)	3 (37.5)
papGII	21 (42.9)	3 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (37.5)	3 (37.5)
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papC	13 (26.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (20.3)	0 (0.0)
Tsh	29 (59.2)	5 (71.4)	1 (50)	1 (100)	4 (80)	0 (0.0)	34 (60.7)	6 (75)
iutA	31 (63.3)	5 (71.4)	2 100)	1 (0.0)	3 (60)	0 (0.0)	36 (64.3)	6 (75)
iroN	46 (93.9)	7 (100)	2 100)	1 (100)	5 (100)	0 (0.0)	53 (94.6)	8 (100)
Iss	18 (36.7)	2 (28.6)	0 (0.0)	0 (0.0)	2 (40)	0 (0.0)	20 (35.7)	2 (25)
ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	42 (85.7)	7 (100)	2 100)	1 (100)	4 (80)	0 (0.0)	48 (85.7)	8 (100)
cva/cvi	28 (57.1)	3 (42.9)	1 (50)	0 (0.0)	5 (100)	0 (0.0)	34 (60.7)	3 (37.5)
	Polymyxins resistant and Susceptible phenotype							
	R (n= 38)	S (n=18)	R (n= 2)	S n=1)	R (n=3)	S (n=2)	R (n=44)	S (n=20)
papGI	10 (26.3)	2 (11.1)	1 (50)	1 (100)	2 (66.7)	0 (0.0)	13 (29.5)	3 (15)
papGII	21 (55.2)*	3 (16.7)*	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (47.7)*	3 (15)*
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papC	13 (34.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (29.5)	0 (0.0)
Tsh	29(76.3)*	5 (27.8)*	1 (50)	1 (100)	3 (100)	1 (50)	33 (75)*	7 (35)*
iutA	31 (81.6)*	5 (27.8)*	2 (100)	1 (100)	2 (66.7)	1 (50)	36 (81.8)*$^{\infty}$	6 (30)*$^{\infty}$
iroN	36 (94.7)	17 (94.4)	2 (100)	1 (100)	3 (100)	2 (100)	41 (93.2)	20 (100)
Iss	18 (47.4)*	2 (11.1)*	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	20 (45.4)*$^{\infty}$	2 (10)*$^{\infty}$
ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	34 (89.5)	15 (83.3)	2 (100)	1 (100)	3 (100)	1 (50)	39 (81.8)	17 (85)
cva/cvi	21 (55.3)	10 (55.6)	1 (50)	0 (0.0)	3 (100)	2 (100)	25 (56.8)	12 (60)

VF: Virulence factor; R: Resistant; S: Susceptible

$^{\infty}$Significant at $P < .05$ (Chi-square statistic); *****Significant at $P < .05$ (t-test).

Table 3.9

Virulence factors in relation to Fosfomycin and Doxycycline resistant phenotype among 64 APEC isolates recovered from broilers' farms in northern Palestine with colibacillosis

VF gene	No. (%) of APEC isolates							
	Fosfomycin-resistant and Susceptible phenotype							
	Group D n= (%)		Group B1 n= (%)		Group B2 n= (%)		Group D/B1/B2 n =(%)	Group D/B1/B2 n =(%)
	R (n=20)	S (n=36)	R (n= 0)	S (n=3)	R (n=2)	S (n= 3)	R (n=22)	S (n=42)
papGI	7 (35)	5(13.9)	0 (0)	2(66.7)	1 (50)	1 (33.3)	8(36.4)	8 (11.9)
papGII	12 (60)	12 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	12 (54.5)*	12 (28.6)*
papGIII	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
papC	8 (40)*	5 (13.9)*	0 (0)	0 (0)	0 (0)	0 (0)	8 (36.4)*	5 (11.9)*
Tsh	13 (65)	21 (58.3)	0 (0)	2 (66.7)	2(100)	2 (66.7)	15 (68.2)	25 (59.5)
iutA	15 (75)	21 (58.3)	0 (0)	3 (100)	1 (50)	2 (66.7)	16 (72.7)	26 (61.9)
iroN	20 (100)	33 (91.7)	0 (0)	3 (100)	2 (100)	3 (100)	22 (100)	39 (92.9)
Iss	5 (25)	15 (41.7)	0 (0)	0 (0)	1 (50)	1 (33.3)	6 (27.3)	16 (38.1)
ompT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
hlyF	18 (90)	31 (86.1)	0 (0)	3 (100)	2 (100)	2 (66.7)	20 (90.9)	36 (85.7)
cva/cvi	9 (45)	22 (61.1)	0 (0)	1 (33.3)	2 (100)	3 (100)	11 (50)	26 (61.9)
	Doxycycline-resistant and Susceptible phenotype							
	R (n= 30)	S (n=26)	R (n= 1)	S (n=2)	R (n=1)	S (n= 4)	R (n=32)	S (n=32)
papGI	6 (20)	6(23.1)	1(100)	1(50)	1 (100)	1 (25)	8(25)	8 (25)
papGII	13 (60)	11 (60)	0 (0)	0 (0)	0 (0)	0 (0)	13 (40.6)	11 (34.4)
papGIII	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
papC	4 (13.3)	9 (34.6)	0 (0)	0 (0)	0 (0)	0 (0)	4 (12.5)	9 (28.1)
Tsh	18 (60)	16 (61.5)	0 (0)	2 (100)	1(100)	3 (75)	19 (59.4)	21 (65.6)
iutA	20 (66.7)	16 (61.5)	1 (100)	2 (100)	1 (100)	2 (50)	22 (68.8)	20 (62.5)
iroN	29 (96.7)	24 (92.3)	1 (100)	2 (100)	1 (100)	4 (100)	31 (96.9)	30 (93.8)
Iss	10 (33.3)	10 (38.5)	0 (0)	0 (0)	0 (0)	2 (33.3)	10(31.25)	12 (37.5)
ompT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
hlyF	27 (85.7)	22 (100)	0 (0)	3 (100)	1 (100)	3 (75)	28 (87.5)	28 (87.5)
cva/cvi	18 (60)	13 (50)	0 (0)	1 (50)	1 (100)	4 (100)	19 (59.4)	18 (56.3)

VF: Virulence factor; R: Resistant; S: Susceptible

*****Significant at $P < .05$ (t-test).

Table 3.10

Virulence factors in relation to Florfenicol and Sulfamethoxazole/trimethoprim resistant phenotypes among 64 APEC isolates recovered from broilers' farms in northern Palestine with colibacillosis

VF	No. (%) of APEC isolates							
	Florfenicol-resistant and Susceptible phenotype							
	Group D n= (%)		Group B1 n= (%)		Group B2 n= (%)		Group D/B1/B2 n =(%)	Group D/B1/B2 n =(%)
	R (n=47)	S (n=9)	R (n= 2)	S (n=1)	R (n=5)	S (n=0)	R (n=54)	S (n=10)
papGI	11(23.4)	1(11.1)	1(50)	1(100)	2 (40)	0 (0.0)	14 (25.9)	2 (20)
papGII	19 (40.4)	5 (45)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	19 (35.2)	5 (50)
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papC	10 (21.3)	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.5)	3 (30)
Tsh	30 (63.8)	4 (44.4)	1 (50)	1 (100)	4 (80)	0 (0.0)	35 (64.8)	5 (50)
iutA	29 (61.7)	7 (77.8)	2 (100)	1 (100)	3 (60)	0 (0.0)	34 (63)	8 (80)
iroN	44 (93.6)	9 (100)	2 (100)	1 (100)	5 (100)	0 (0.0)	51 (94.4)	10 (100)
Iss	18 (38.3)	2 (2.2)	0 (0.0)	0 (0.0)	2 (40)	0 (0.0)	20 (37)	2 (20)
ompT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	43 (91.5)*	6 (66.7)*	2 (100)	1 (100)	4 (80)	0 (0.0)	49 (90.7)	7 (70)
cva/cvi	26 (55.3)	5 (55.6)	1 (50)	0 (0.0)	5 (100)	0 (0.0)	32 (59.3)	5 (50)
	Sulfamethoxazole/trimethoprim resistant and Susceptible phenotype							
	R (n=44)	S (n=12)	R (n=2)	S (n=1)	R (n=4)	S (n=1)	R (n=50)	S (n=14)
papGI	10 (22.7)	2 (16.7)	1 (50)	1 (100)	1 (25)	1 (100)	12(24)	4 (28.6)
papGII	20 (45.5)	4 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (40)	4 (28.6)
papGIII	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
papC	11 (25)	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11(22)	2 (14.3)
Tsh	28 (63.6)	6 (50)	1 (50)	1 (100)	3 (75)	1 (100)	32 (64)	8 (57.1)
iutA	30 (68.2)	6 (50)	2 (100)	1 (100)	2 (50)	1 (100)	34(68)	8 (57.1)
iroN	42 (95.5)	11 (68.8)	2 (100)	1 (100)	4 (100)	1 (100)	48(96)	13 (92.9)
Iss	14 (31.8)	6 (50)	0 (0.0)	0 (0.0)	2 (50)	0 (0.0)	16 (32)	6 (42.9)
ompT	0 (0.0)	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
hlyF	41 (93.2)*	8 (66.7)*	2 (100)	1 (100)	3 (75)	1 (100)	46 (92)*	10 (71.4)*
cva/cvi	25 (56.8)	6 (50)	1 (50)	0 (0)	4 (100)	1 (100)	30(60)	7 (50)

VF: Virulence factor; R: Resistant; S: Susceptible

*****Significant at P < .05 (t-test)

Chapter Four

Discussion

E.coli can grow in various hosts and settings due to its incredibly diverse DNA. These bacteria can survive as either pathogens or commensal organisms since they have evolved alongside humans and have colonized a variety of hosts (Ovi et al., 2023). More recent studies have highlighted the critical role of broiler breeders as APEC infection reservoirs through vertical transmission to chicken and subsequent horizontal transmission between chicken. Furthermore, all poultry species must be continuously tracked and monitored for APEC strains because of their continually evolving genetic diversity (Joseph et al., 2023). In addition, the detection of APEC isolates as a foodborne pathogen that contaminates chicken during meat processing can be transmitted to the consumers posing a risk to them. Therefore, in the future, our research on the genetic diversity of APEC isolates recovered from infected chicken obtained from broiler farms in Palestine may be utilized to reduce consumer risk, economic losses due to chicken mortality, decreased egg and meat production, and antibiotic therapy expenses. To the best of our knowledge, this is the first research to describe APEC isolates from broiler breeders in Palestine that have colibacillosis, and it further provides data on their genotypic-virulence characteristics, phylogenetic groups as well as antimicrobial resistance patterns.

The findings of this study showed a high detection rate of APEC strains (100%) in chicken recovered from broilers' farms in northern Palestine using classical cultural methods and molecular methods (PCR). Results of this study showed a high detection rate similar to other studies previously published. The detection rate of APEC strains ranged from 77%-90.4% in Nepal (Thapa & Chapagain, 2020; Bista et al., 2020; Bhattarai et al., 2023), 100% in India (Dadheech et al., 2016) and 86.7% in Algeria (Halfaoui et al., 2017).

The current study showed that the predominant APEC strains isolated from poultry that had been clinically diagnosed with colibacillosis belonged to phylogenetic group D. Extraintestinal pathogenic *E. coli*, including APEC, is classified as belonging to phylogroups B2 and D, according to several studies have been published previously (Logue et al., 2017; Joseph et al., 2023; Ovi et al., 2023). Through phylogenic examinations, this *E. coli* was found to have a noticeable overlap with the phylogroup of *E. coli* that infects people. This overlap suggests a significant zoonotic potential for APEC, which may result from parallel evolution and development in both hosts from a

shared parent lineage (Ovi et al., 2023). The findings corresponded with previous research on APEC phylogenetic groupings (Kim et al., 2020; Thomrongsuwannakij et al., 2022).It was reported that the common APEC isolates recovered from commercial boilers belonged to group D (41.1%) detected by Thomrongsuwannakij et al., (2022) and (46.8%) by Kim et al.,(2020).This study supported the findings of Adwan & Issa, (2015) investigation of uropathogenic *E. coli* recovered from human urine in Palestine, which indicated that the majority of isolates (72%) belonged to phylogenetic group D. The findings of this study were in contradiction with the results of other study concerning phylogenetic groups in APEC (Joseph et al., 2023),which showed that most APEC isolates of colibacillosis recovered from broiler breeders belonged to phylogenetic group B2, which had a prevalence rate of 71.4%, while group D had a 25% rate Data published previously (Hussein et al., 2013), showed that most APEC isolates of colibacillosis recovered from broiler breeders belonged to phylogenetic A (54.2%). Data published recently (Thomrongsuwannakij et al., 2022), revealed that phylogenetic group A accounted for the majority of APEC isolated from local chicken. (Afayibo et al., 2022), showed that group D had a prevalence rate of 20.00%, while phylogenetic groups B2 and A were the most prevalent, with prevalence rates of 29.57% and 26.96%, respectively. Other phylogenetic groupings in the current study with prevalence rates of 1.5%, 4.6%, and 7.8% for phylogenetic groups A, B1, and B2, respectively.

These findings were in contrast to other studies concerning the prevalence of phylogenetic groups in APEC (Thomrongsuwannakij et al., 2022). According to Thomrongsuwannakij et al., (2022), APEC isolates recovered from commercial boilers belonged to groups A, B1, and B2, with prevalence rates of 5.6%, 22.2%, and 31.1%, respectively. For these isolates obtained from local chickens, the prevalence rates were 35.7%, 26.2%, and 30.9% *for phylogenetic groups A, B1, and B2, respectively. In addition, the prevalence rates conflicted with those published previously (Joseph et al., 2023), which showed that the prevalence of A, B1 and B2 were 0.0%, 3.6% and 71.4, respectively. The results of the current study were incompatible with those reported recently (Kim et al., 2020), which showed that prevalence rates were 22.8% for phylogenetic groups A and B1. However, the prevalence rate for group B2 was similar to this study (7.6%). Also, the prevalence rates of the current research conflicted with newly published data (Afayibo et al., 2022), which showed prevalence rates of 26.96%, 18.26% and 29.57% for phylogenetic groups

A, B1 and B2, respectively. Furthermore, the study carried out by (Afayibo et al., 2022), showed 5.22% of the isolates were not assigned to any phylogenetic groups.

The broiler industry has serious problems associated with both economic and welfare because of APEC. Antimicrobial growth promoter supplementation is the main strategy used to avoid APEC infections. The antimicrobial resistance among APEC isolates has increased dramatically worldwide (Azam et al., 2019; FR et al., 2019; Kim et al., 2020; Ievy et al., 2020; Johar et al., 2021;Afayibo et al., 2022; Thomrongsuwannakij et al., 2022). In the current study, a high resistance rate was detected to Amoxicillin, Enrofloxacin, Ceftiofur, Ceftriaxone, Norfloxacin, Florfenicol, Ciprofloxacin, Gentamicin, Sulfamethoxazole/trimethoprim and Cephalexin. These results were more or less similar to the findings of other studies published previously from different parts of the world (Azam et al., 2019; FR et al., 2019; Ievy et al., 2020; Johar et al., 2021; Afayibo et al., 2022). These antimicrobials could be the most commonly utilized in animals and poultry, which might account for the isolates' high resistance to these antibiotics. Isolates of APEC showed moderate resistance rates to florfenicol and gentamicin (Afayibo et al., 2022), and all native chicken APEC isolates were susceptible to gentamicin (Thomrongsuwannakij et al., 2022). In other studies, most APEC isolates were susceptible to gentamicin (Ievy et al., 2020; Joseph et al., 2023). APEC isolates revealed a moderate resistance rate against Enrofloxacin (Ievy et al., 2020).

Commercial chicken APEC isolates showed a moderate resistance rate against sulfamethoxazole/trimethoprim (Thomrongsuwannakij et al., 2022). In this study, APEC isolates showed a moderate resistance rate against Neomycin, Polymyxins B and Doxycycline. In a recently published report (Afayibo et al., 2022), APEC isolates showed a highly resistant rate to Doxycycline. Polymyxins E (Colistin) and Fosfomycin showed a low resistance rate in this study. This is consistent with results published previously (Ievy et al., 2020), which showed low susceptibility was detected against colistin sulfate. In addition, all commercial chicken APEC isolates were sensitive to colistin (Thomrongsuwannakij et al., 2022). This is inconsistent with results published previously (FR et al., 2019), which showed moderate susceptibility of APEC against colistin sulfate. The results of this research were incompatible with those reported recently, which showed that APEC isolates recovered from healthy and non-healthy chickens exhibited a high resistance rate against Fosfomycin (Johar et al., 2021). Future issues with APEC

strains come from their resistance to antimicrobial agents. It would be preferable to control avian colibacillosis via vaccination (Bhattarai et al., 2023).

In this research, fifty-eight resistance patterns were observed in the 65 APEC isolates and 7 clusters depended on the resistance/sensitivity of 65 APEC strains to the antibiotics used in this study, but the clustering process using dendrogram is independent of phylogenetic groups. In addition, Clusters C1, C2, C3 and C4 can be divided into 2 or 3 sub-clusters indicating a high diversity of resistance patterns in APEC strains in these broilers' farms in northern Palestine. These results also showed that all APEC isolates were MDR. The results of this study are in agreement with other studies published previously which showed that all isolates or most of the examined isolates were multidrug-resistant (Hussein et al., 2013; Azam et al., 2019; FR et al., 2019; Ievy et al., 2020; Afayibo et al., 2022). This may be due to the extensive and inappropriate use of antimicrobial drugs, such as overuse, prophylactic usage, feed additives or growth promoter use, which might contribute to the rise in antimicrobial resistance (Thomrongsuwannakij et al., 2022; Joseph et al., 2023).

Antimicrobial resistance in humans and the use of antibiotics in food animal production have been shown to be closely associated in several research. The clinical utility of antibiotics in humans and animals is thought to be threatened by antibiotic misuse in livestock. As a result, efforts have been made to avoid the use of highest priority clinically important antimicrobials in food production animals in order to keep their effectiveness in clinical settings. Unfortunately, there aren't enough suitable highest priority clinically important antimicrobial substitutions for many health conditions (Mehat et al., 2021). Antimicrobial resistance in chickens is a common problem in Palestine as well as other developing countries. The result of the current study is inconsistent with results published recently which showed that only 25% of isolates were MDR (Gambi et al., 2022).

Plasmids are the primary source of virulence factors that contribute to the bacterial characteristics required for establishing an APEC infection. Therefore, it is frequently not possible to identify APEC using phylogenetic classification according to chromosomal markers. Furthermore, the relationship between phylogenetic taxonomy and pathotype is made more complicated by the fact that *E. coli* can acquire virulent plasmids from other bacteria (Ovi et al., 2023). Several virulence determinants that are highly common only in APEC isolates have been found in previous studies. Functions of these genes may be

necessary for APEC infections in poultry to develop. As a result, APEC may be differentiated from commensal, intestinal, environmental, and other extraintestinal *E. coli* using these genes When evaluating the virulence of APEC isolates, the following Virulence genes (episomal *iss*, *tsh*, *iroN*, episomal *ompT*, *iutA*, *cvaC*, *hlyF*, *iucD*, *papG* allel (II/III), and *papC*) should be taken into consideration (Ovi et al., 2023).

Plasmid-linked virulence genes obtained by horizontal gene transfer are found in the majority of APEC isolates. There isn't a single particular virulence gene that differentiates APEC apart from other strains of *E. coli*. However, certain plasmid-carried virulence-associated genes, including *hlyF*, *ompT*, *iroN*, *iss*, and *iutA*, were shown to be often present in APEC strains. These genes may be used to differentiate non-pathogenic *E. coli* strains from APEC strains (T. J. Johnson et al., 2008). There is a wide range in the proportion of virulence factors analyzed in this study, from 0.0% to 93.8%. The virulence genes for *iroN*, *hlyF*, *iutA*, *Tsh*, *ColV*, *papGII*, *iss*, *papGI*, *papC*, *papGIII*, and *ompT* were found to be 93.8%, 86.2%, 64.6%, 61.5%, 56.9%, 36.9%, 35.4%, 24.6%, 20.0%, 0.0%, and 0.0% prevalent in the current study, respectively. These findings were more or less similar to the results of other studies published previously from different parts of the world (Hussein et al., 2013; Kim et al., 2020; Johar et al., 2021; Afayibo et al., 2022; Awawdeh et al., 2022; Gambi et al., 2022; Joseph et al., 2023; Ovi et al., 2023).

The most frequently occurring genes among the APEC isolates in the current study were discovered to be those encoding for iron acquisition *iroN* (93.8%) and *iutA* (64.6%), and toxin production *hlyF* (86.2%). These genes are more frequently related to highly pathogenic APEC and are associated with the ColV plasmid. (Joseph et al., 2023). It was reported that the following five genes *iroN*, *ompT*, *hlyF*, *iss* and *iutA* are associated with the ColV plasmid, indicating that these APEC isolates, harbored plasmid pathogenicity islands (PAIs) (T. J. Johnson et al., 2006; Hussein et al., 2013; Joseph et al., 2023). These genes have been observed to be more commonly linked to extremely pathogenic APEC (T. J. Johnson et al., 2008).

The findings of this investigation, however, disagreed with those of a previous study (Joseph et al., 2023), in this study *iss* and *ompT* genes were not detected in all APEC isolates. However, Joseph et al., (2023), demonstrated that the *iss* and *ompT* genes were linked to the ColV plasmid, were found to be more frequently associated with highly pathogenic APEC strains, and had the highest prevalence among APEC isolates. In this

study, according to the method described by Joseph et al., (2023), all isolates (64 isolates) belonged to groups D, B1 and B2 and had genes that are primarily associated with highly pathogenic APEC strains, however, the strain belonged to group A had only one gene (*iss*) that may be a low pathogenic or a nonpathogenic APEC strain. APEC isolates must be characterized to fully understand the pathogenesis of colibacillosis and develop efficient preventative and control measures for the illness. Results of this study, suggest that the PAI is not most frequently found on ColV plasmids in APEC isolates but might occur in another genomic location and is not necessary to have the 5 genes (*iroN*, *ompT*, *hlyF*, *iss* and *iutA*) together. PAI may be seen in APEC isolates in alternative sites such as on ColBM plasmids (T. J. Johnson et al., 2006). Diagnostic approaches to recognizing APEC isolates are based on identifying several virulence genes of *E. coli* (Azam et al., 2019). According to the method described by Johar et al., (2021), the APEC strain is considered pathogenic if it has more than 4 of the detected genes.

Based on this method, only 55.4% of strains in this study are considered pathogenic APEC. The result of this study is consistent with that reported previously (Azam et al., 2019), which showed that 42.6% and (Gambi et al., 2022) 56% of isolates were considered as more virulent APEC, based on the genetic criteria of possessing more than 4 virulence factors. However, compared to these three ColV plasmid genes (*iroN*, i*utA*, and *hlyF*), the gene *cva/cvi*, which is part of the ColV plasmid, was shown to have a lower prevalence than the prevalence of *iroN*, i*utA*, and *hlyF* genes. This was unusual, but it has also been reported previously in turkeys with cellulitis (De Oliveira et al., 2020) and from APEC isolated from broiler breeders with colibacillosis in Mississippi, USA (Joseph et al., 2023). More research is necessary in this regard. Also, results of this study showed that, 34 virulence gene patterns were observed among the 65 APEC isolates with *Tsh*, *iutA*, *iroN*, *hlyF* and *cva/cvi* pattern being the most predominant (12.3%). In addition, 6 clusters depended on the presence/absence of virulence factors in 65 APEC strains used in this study, but the clustering process using dendrogram is independent of phylogenetic groups, each of these clusters can be divided into 2 or more sub-clusters. These results indicating that APEC strains isolated from broilers' farms in northern Palestine had a high diversity of virulence genes. In the future, information about virulence genes can be used for colibacillosis diagnosis and also as a potential vaccine candidate.

In the current study, the *hlyF* gene prevalence was 88.9%, 87.5%, 85.7%, 81.8%, 90.9%, 87.5%, 90.7% and 92% for isolates resistant to fluoroquinolones, Cephalosporins, Aminoglycoside, Polymyxins, Fosfomycin, Doxycycline, Florfenicol and Trimethoprim/Sulphamethoxazole, respectively. The *iutA* gene was the most common prevalence among strains resistant to Polymyxins. In this study, significant associations ($P < 0.05$) between specific virulence factor content and phenotypic resistance were detected among clinical APEC isolates. These include *iutA* and *iss* Polymyxins resistance. In study published recently, significant associations ($P < 0.05$) between specific VG content and phenotypic resistance were detected among clinical APEC isolates. These included: *iroN* with ampicillin resistance and *iss-iutA-ompT-hlyF-iroN* with gentamicin resistance for clinical APEC isolates (Awawdeh et al., 2022).

In this study, according to the distribution of virulence factors based on the phylogenetic groups, results showed that B2 group (100%) had a higher prevalence of *cva/cvi* virulence factor than group B1 (33.3%) ($P < 0.05$). In a recent study, certain phylotypes were associated with certain virulence factors, as demonstrated by the finding that groups B1 and D had a higher prevalence of *papC* and *vat* virulence factors than groups A and B2. The virulence determinants *fyuA*, *iucD*, and *cva/cvi* were widely distributed in groups D (84.78%), B1 (80.95%), and A (80.65%), respectively. Furthermore, the *neuC* and *vat* genes were lower in group A strains, although phylotype A strains were the main group with the gene *ibeA* (Afayibo et al., 2022).

4.1 Conclusion

These results serve as an outline for further investigations into the pathophysiology of APEC and the development of efficient intervention plans for both the avoidance and management of APEC in broiler breeders and broiler farms. Furthermore, it was concluded that Polymyxins E (Colistin) and Fosfomycin will be the first antimicrobial agents of choice to combat infections caused by APEC in Palestine. The determination of virulence genes of APEC strains and antibiotic resistance is essential to understand its pathogenesis, antimicrobial therapy, and the development of measures to control colibacillosis such as vaccination, feed hygiene, housing management strategy and others. Controlling the permissible level of Iron, both in water source and feed for broiler breeder and broiler farms, as it is an important element for the proliferation of *E.coli* .

Maintaining APEC infections under control is essential for public health, especially when MDR genes are present in APEC isolates. Additionally, APEC isolates can pass on resistance and virulence genes to dangerous bacteria such as *E. coli* that are particular to humans.

List of Abbreviations

Abbreviation	Meaning
iss	Increased serum survival gene (Protectins)
Tsh	Temperature-sensitive hemagglutinin gene (Adhesins)
iutA	Ferric aerobactin receptor gene; iron transport (Iron acquisition)
iroN	Catecholate siderophore receptor gene (Iron acquisition)
hlyF	Hemolysin F gene (Toxins)
hlyA	α-hemolysin gene
hlyE	Haemolysin E gene
ompT	Outer membrane protease gene (Protectins)
papG	P fimbrial adhesin gene
papGI	Pyelonephritis-associated pili allele I (Adhesins)
papGII	Pyelonephritis-associated pili allele II (Adhesins)
papGIII	Pyelonephritis-associated pili allele III (Adhesins)
papC	encoding P fimbriae
papA	P fimbrial adhesins
TspE4C2	antibiotic resistance fragment
cva/cvi	Colicin V plasmid operon genes
yjaA	polypeptide stress response protein
phoA	nonspecific phosphomonoesterase
iucC	aerobactin synthesis
ChuA	Outer membrane hemoglobin receptor protein
cvaC	Colicin V
iucD	aerobactin gene(encodes lysine:N6-hydroxylase)
fimH	type 1 fimbriae Adhesins
traT	serum resistance- outer membrane protein
OmpA	outer membrane protein
afa	A fimbrial Adhesins
PhoPQ	two-component regulatory system
OmpF	major outer membrane protein
Cvi	encoding the colicin V
aerJ	Aerobactin
papEF	pilus associated with pyelonephritis
ibeA	invasion of brain endothelium
ibeB	invasion protein

Tia	toxigenic invasion locus
gimB	genetic island linked with newborn meningitis
Bfr	Bacterioferritin
mntH	iron and manganese transporter
ireA	iron-regulated virulence gene
irp2	iron repressible protein
feoB	ferrous ion transporter
fyuA	Yersiniabactin
eitABCD	putative iron transporter
fepC	ferric enterobactin transporter
iroBCDEN	Salmochelin
tolC	outer membrane efflux protein
entE	enterobactin synthesis genes
entS	enterobactin transport genes
Pic	serine protease autotransporter
espC	serine protease
ace4/35	acetylcholine esterase
stx2f	Shiga toxin variant
hlyE	putative avian hemolysin
Vat	vacuolating autotransporter toxin
cdtS	cytolethal distending factor
Sat	secreted autotransporter toxin
QS	Quorum-Sensing System
ptsI	phosphotransferase system
DotU	organelle trafficking protein
IcmF	intracellular multiplication factor
VrgG	secreted protein
CpxA	envelope stress response system
Hcp	hemolysis co-regulation protein
(H)	Flagellar
(K)	Capsular
(O)	Somatic
APEC	Avian pathogenic *E. coli*
CLSI	Clinical & Laboratory Standards Institute
DAEC	diffusely adherence *E. coli*
DEC	diarrheagenic *E. coli*

DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphates
E. coli	Escherichia coli
EHEC	enterohemoragic *E. coli*
EIEC	entero-invasive *E. coli*
EPEC	enteroaggregative *E. coli*
ETEC	enterotoxigenic *E. coli*
ExPEC	extraintestinal pathogenic *E. coli*
HAP	hospital-acquired pneumonia
HUS	Hemolytic-uremic syndrome
LPS	Lipopolysaccharides
MDR	Multidrug resistance bacteria
NMEC	newborn meningitis
PCR	polymerase chain reaction
SEPEC	sepsis associated *E. coli*
SSI	surgical site infection
Taq DNA polymarase	Thermous aquaticus DNA polymease
UPEC	uropathogenic *E. coli*
UTI	urinary tract infection
UTI	urinary tract infections
VF	virulence factor
VFGs	virulence factor genes
TSB	Tryptone soy broth
EMB	Eosin Methylene Blue
TSI	Triple Sugar Iron
SIM	Sulfide Indole Motility
MR-VP	Methyl red-Voges Proskauer
TTC	triphenyl tetrazolium chloride
EDTA	Ethylenediaminetetraacetic acid
H2S	hydrogen sulfide
MgCl2	Magnesium chloride
MR	Methyl Red
Tris	Commom ph buffer
VP	Voges-Proskauer
$\chi 2$	Chi-square test

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Appendices

Appendix A

Tables

Table 3.1

Occurrence of phylogenetic groups among 65 APEC isolates

Group		**Total APEC isolates (65)**		
	Sub-group	No. of isolates	Sub-group %	Group %
A	A0	1	1.5	1.5
B1	B1	3	4.6	4.6
B2	B22	4	6.3	
	B23	1	1.5	7.8
D	D1	9	13.8	
	D2	47	72.3	86.1
Total		65	100	100

Table 3.3

The antimicrobial resistance patterns of APEC isolates collected from broiler farms in northern Palestine

	Resistance pattern	No. of multi-drug resistant isolates
1	FFC,CL,CN,AX,NOR,CIP,ENR,CRO,STX.	1
2	FFC,CL FUR,AX,NOR,CIP,ENR.	1
3	FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
4	N,CT,PB,DO,FFC,CN,FUR,AX,ENR,CRO,STX.	1
5	N,FO,PB,FFC,CL,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
6	PB,DO,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
7	N,CT,PB,,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1
8	N,PB,DO,FFC,CL,CN,FUR,AX, CIP,ENR,CRO,STX.	1
9	N,FO,PB,DO,FFC, CL,AX,NOR,CIP,ENR,CRO,STX.	1
10	DO,AX,NOR,CIP,ENR,CRO,STX.	1
11	N,PB,FFC,CL,CN,FUR,AX,ENR,CRO,STX.	1
12	N,FO,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
13	N,FO,PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	3
14	N,CT,PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1
15	N,CT,FO,PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1
16	N,CT,FO,PB,FFC,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
17	N,CT,FO,FFC,CL,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
18	CT,PB,DO,CL,CN,FUR,AX,ENR,CRO.	1
19	N,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	2
20	N,FUR,AX,CRO,STX.	1
21	N,FO,PB,DO,FFC,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	2
22	DO,FFC,CL,FUR,AX,CIP,ENR,CRO,STX.	1
23	CT,FO,PB,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
24	N,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
25	N,FO,PB,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
26	PB,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1
27	PB,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
28	PB,DO,FFC,CL,FUR,AX,CIP,ENR,CRO,STX.	1
29	N,FO,PB,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
30	N,FO,PB,DO,CN,FUR,AX,NOR,ENR,CRO,STX.	1
31	PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	2
32	AX,NOR,CIP,ENR,CRO.	2
33	DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
34	FO,PB,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
35	FO, PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
36	CT,PB,FFC,CN.FUR,AX,NOR,CIP,ENR,CRO,STX.	1
37	DO,FFC,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
38	PB,DO, FFC, CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
39	PB,FFC,CL, FUR,AX,NOR,CIP,ENR, STX.	1
40	N,CT,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	2
41	CT,FO,DO,FFC,CL,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
42	N,CT,DO ,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1
43	N,CT,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,STX.	1
44	N ,CT,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,STX.	1
45	N,FFC,CL,CN,FUR,AX,NOR,CIP,ENR.	1
46	FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
47	N ,DO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO.	1

48	N,FO,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	2
49	N,FO,DO,FFC,CN,FUR,AX,NOR,CIP,ENR, STX.	1
50	N,PB,DO,FFC,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
51	N,PB, FFC,CN,FUR,AX ,NOR,ENR,CRO,STX.	1
52	N,PB.FFC,CL,CN,FUR,AX,NOR,ENR,CRO,STX.	2
53	N,CT,FO,PB,DO,FFC ,FUR,AX ,NOR,CIP,ENR,CRO,STX.	1
54	N,CT,PB,FFC,CN,FUR,AX,NOR,CIP,ENR,CRO,STX.	1
55	N,PB,FFC,CL,CN,FUR,AX,NOR,ENR,CRO,STX.	1
56	DO,FFC,CL,CN,FUR,AX,ENR,CRO.	1
57	N,FO,PB,FFC,CL,CN,FUR,AX,NOR,CIP,ENR,STX.	1
	Total	65

*Neomycin sulfate, N; Colistin sulphate, CT; Polymyxin B, PB; Doxycycline, DO; Florfenicol, FFC; Cephalexin, CL; Gentamicin, CN; Ceftiofur, FUR; Amoxicillin, AX; Norfloxacin, NOR; Enrofloxacin, ENR; Ciprofloxacin, CIP; Ceftriaxone, CRO; Sulfamethoxazole/Trimethoprim, STX; Fosfomycin, FO.

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

المقاومة للمضادات البكتيرية والتوصيف الجزيئي لعزلات بكتيريا الإشريكية القولونية الممرضة للطيور المستخرجة من مزارع الدجاج اللاحم في شمال فلسطين

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الإنتاج الحيواني، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2024

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الملخص

الخلفية :يَحدثُ مرضُ داءِ العصياتِ القولونيةِ بِسَببِ الإشريكيةِ القولونيةِ المُسَبِبَةِ لِأمراضِ الطُيور(APEC) ، مما يُؤدي إلى خسائرَ كبيرةٍ لقطاعِ الدواجن ويُظهِرُ إمكانية الانتقالِ من الحيوانِ للإنسانِ ويعملُ كمصدرٍ لمقاومةِ المضاداتِ الحيويةِ والجيناتِ المُمرِضةِ للبكتيريا الإشريكيةِ القولونية الأخرى.

الأهداف: يهدفُ هذا العمل إلى تقييمِ المجموعات التطورية والعوامل المسببة للمرض والنمط الظاهري للمقاومة في مجموعةٍ من السلالاتِ الإشريكيةِ القولونية المسببة للأمراض الطيورِ (APEC) المعزولةِ من مزارع الدجاج اللاحم في شمالِ فلسطين. بالإضافة إلى توضيح ما إذا كانت العوامل المسببة للمرض مرتبطة بشكل مباشر بمقاومة المضادات الحيوية أو تعتمد بدلاً من ذلك على توزيع المجموعة التطورية.

المنهجية: تم عزلُ ما مجموعهِ 65 عزلَةٍ من الدجاجِ المصابِ بمرضِ داء العُصيَّاتِ القولونيةِ من مزارعِ الدجاج التي تقعُ في المنطقةِ الشماليةِ من فلسطين، خلالَ الفترةِ من أيارَ/مايو إلى تموز/يوليو 2024.

تَم تحديدُ السلالاتِ من خلالِ التقنيات الكلاسيكية والجُزَيئيةِ. تم الكشفُ عن مقاومةِ المضاداتِ الحيويةِ باستخدام طريقة الانتشارِ القُرصي. تم الكشفِ عن المجموعات التطوريةِ والجيناتِ المُمرضة لعَزلاتِ الإشريكيةِ القولونيةِ المُسَببةِ لأمراضِ الطُيورِ بِواسِطَةِ تِقَنيةِ سِلسِلةِ تفاعلُ البلمرةِ المتعدد.

النتائج :أظهرت هذهِ الدراسةُ معدلَ اكتشافٍ عالٍ من السلالاتِ الإشريكية القولونية المُسَبِبةِ لأمراضِ الطيور (100%) (APEC) في الدجاج. تنتمي معظمُ السلالاتِ الإشريكيةِ القولونية المُسببةِ لأمراضِ الطيورِ (APEC) (65/56 86.2٪) إلى المَجموعةِ D .السلالاتُ الأخرى تَنتمي للمجموعةِ B2 (5\65, 7.7٪), B1 (3\65, 4.6٪), A1 (1\65, 1.5%). تتراوحُ مقاومةُ المضاداتِ الحيويةِ من 27.7٪ للبولمكسين E(الكلستين) الى 100٪ للأموكسيسيلين. كان البوليميكسين E (كوليستين) و الفوسفوميسين أكثرَ الأدويةِ فعاليةً. وُجِدَ أنَ الجينَ المُمرضُ الأكثرَ شيوعاً هو (ironN) الذي تَم اكتِشافَهُ في 61 عَزلَةٍ (93.8%). في حينِ أنَ 65 (86.2٪)، 42 (64.6٪)، 40 (61.5٪)، 56 (86.2٪)، 37 (56.9٪)، 24 (36.9٪)، 23 (35.4٪)، 16 (24.6٪)، 13 (20.0٪)، 0(0.0٪) و 0 (0.0٪) كانتِ العزلاتُ إيجابيةً ل *hlyF, iutA, Tsh, ColV, papGII, Iss, papGI, papC, papGIII* و *ompT* جينٍ على التوالي. تُظهِرُ سلالاتِ الإشريكية القولونية المسببة لأمراضِ الطيور (APEC) في فلسطينِ مجموعةٍ واسعةٍ من أنماطِ المُقاوَمَةِ والتَبايُنِ الجيني.

الخلاصة: هذه النتائج بمثابة الخطوط العريضة لتطوير خطط التدخل الفعالة لإدارة عدوى السلالاتِ الإشريكية القولونيةِ المُسببةُ لأمراضِ الطيور في مزارع أمهات الدجاج اللاحم ومزارع الدجاج اللاحم. السَيطرةُ على عدوى السلالاتِ الإشريكية القولونيةِ المُسببةُ لأمراضِ الطيور (APEC) أمرٌ ضروريٌ للصحةِ العامة، وخاصةً أنَ عَزلاتِ سلالات الإشريكية القولونية المسببة لأمراضِ الطيور(APEC) يُمكِنُ أن تنقلَ المقاومةَ والجيناتِ المُمرِضةِ إلى البكتيريا الخطيرةِ مثلَ الإشريكيةِ القولونيةِ التي هي خاصةٌ بالبشر .

الكلمات المفتاحية: سلالاتُ الإشريكيةُ القولونيةُ المسببةُ للأمراضِ الطيور، مرض داء العصيات القولونية ، الجينات الممرضة، مقاومة المضادات الحيوية، المجموعات التطورية.