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RESEARCH ARTICLE

Typing Beta-Lactamase-Producing Avian Pathogenic *Escherichia coli* Isolates Recovered from Broiler Farms in Northern Palestine

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ABSTRACT

Avian pathogenic *E. coli* (APEC), responsible for colibacillosis in chickens, is a very important economical infectious bacterium. To treat, control and manage this wide spread infection in chicken production, antimicrobial agents are widely used. In addition, antimicrobial agents are also used to stimulate growth. Antimicrobial resistance in APEC can progress and spread as a result of indiscriminate use of these antimicrobial agents, in addition to poor antibiotic selection, misuse, and overuse. This study was designed to identify extended-spectrum beta-lactamases (ESBLs), metallo-β-lactamases (MBL), integrons, and investigate genetic heterogeneity among 65 APEC strains isolated from necropsies, with clinical signs of colibacillosis. The samples were obtained from broiler farms in Northern Palestine. Multiplex PCR approach was employed to detect ESBL genes and integrons, while RAPD-PCR was utilized to assess genetic diversity among the isolates. The results revealed that the incidence of ESBL genes was 100, 44.6, 1.6, 0.0 and 0.0% for *blaTEM*, *blaCTX*, *blaOXA*, *blaKPC* and *blaSHV*, respectively. The MBL genes were found in 72.3, 3.1, and 0.0% for the *blaSIM*, *blaVIM*, and *blaSPM*, respectively. In all, 100, 35.4 and 0.0% of isolates had the *intI1*, *intI2* and *intI3* genes, respectively. Integron, ESBL, and MBL gene patterns, as well as RAPD-PCR patterns, revealed that these isolates were genetically diverse. The β-lactamase genes test results revealed that broiler chicken products in Palestine may serve as a reservoir for these genes, posing a public health danger. As a result, the careful use of antimicrobials and full surveillance for resistant strains on chicken farms and hatcheries are crucial in limiting the selection and dissemination of extremely dangerous APEC strains.

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INTRODUCTION

Avian Pathogenic *Escherichia coli* (APEC) is recognized as an extraintestinal pathogenic *Escherichia coli* (ExPEC). The APEC pathogen affects turkeys, as well as fast-growing broiler chickens, laying hens, and breeding chickens. Although APEC can often be an opportunistic pathogen or a secondary pathogen after respiratory virus infection or because of insufficient medical treatment or poor management, it can also be a primary pathogen in flocks that are healthy (Watts and Wigley, 2024; Chenouf *et al.,* 2025). The APEC can cause colibacillosis in all ages of birds, a spread APEC infection that can result in significant morbidity and mortality, decreased productivity of meat and egg hatching rates, and carcass rejection at slaughterhouses (Bhattarai *et al*., 2023). Therefore, APEC poses a global threat to bird welfare and food safety (Mehat *et al*., 2021) where the worldwide chicken industry has suffered financial and economic losses as a result (Bhattarai *et al*., 2023). Peritonitis, air sacculitis, polyserositis, omphalitis, salpingitis, cellulitis, coligranuloma, egg peritonitis, pericarditis, perihepatitis, septicaemia and enlarged head syndrome are among the most common symptoms of avian colibacillosis (Misumi *et al*., 2023; Watts and Wigley, 2024).

Beta-lactamases are frequently categorized using two systems: the Bush–Jacoby–Medeiros functional classification and the Ambler molecular classification. The former approach divides these enzymes into four groups (A-D) according to their primary amino acid sequence.

Class A, C, and D enzymes contain an active site serine known as serine β-lactamases, but class B enzymes are metallo-enzymes that frequently need a divalent cation (Zn^{+2}) to promote function. The functional characteristics of enzymes, such as the profiles of substrates and inhibitors, determine the later functional approach. Beta lactamases, especially extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamases, are of particular importance and represent an important problem for clinical therapeutics as these enzyme-producing organisms confer resistance to many antibiotics, leaving a restricted selection of therapeutic agents (Adwan and Abu Jaber, 2016). Both chromosomally mediated β-lactamases that occur spontaneously and plasmid-mediated β-lactamases that are acquired by plasmid transfer (plasmid-mediated resistance) are encoded by the same genes. Most genes that encode ESBL are found on transposons or plasmids that have insertion sequences with other resistance genes. Because of this, they can spread rapidly and can result in resistance to a wide range of antimicrobials, such as sulphonamides, aminoglycosides, trimethoprim, tetracyclines, fluoroquinolones, and chloramphenicol (Husna *et al*., 2023). The ESBL-producing *E. coli* strains recognized from humans and animals across the world have a variety of ESBL genes (Ewers *et al*., 2012; Adwan and Abu Jaber, 2016; Saliu *et al*., 2017). Globally, the frequency of *Enterobacteriaceae* that produce ESBL is rising (Khadka *et al*., 2023). These bacteria are resistant to most β-lactam antimicrobial medications, particularly third and fourth generation cephalosporins, leaving treatment choices extremely restricted.

Based on genotypic analysis between ExPEC and APEC, many reports suggest that APEC may serve as a source of genes that could be transferred to human ExPEC strains (Kang *et al*., 2022; Yoon and Lee, 2022). The emergence of phenotypic drug-resistant bacteria through gene mutations or quick horizontal transmission of resistance genes by mobile genetic elements, including plasmids, gene cassettes and transposons to various *Enterobacteriaceae* including *E. coli* strains is known to be caused by the abuse or overuse of antibiotics in both human and animal populations (Tseng *et al*., 2023; Saeed *et al*., 2023). The effective transmission of ESBL genes, mainly *blaCTX-M*-encoding genes, is primarily due to their presence on conjugative plasmids that may co-exist with other antibiotic resistance genes, causing resistance to other several antibiotic classes, such as aminoglycosides and fluoroquinolones (Chenouf *et al*., 2025). Humans may acquire ESBL-producing *E. coli* through direct contact with the poultry reservoir or through the food chain (Chenouf *et al*., 2025). The use of antimicrobial medications for growth promotion, enhanced feed efficiency, and prophylaxis continues to be legal in several countries. Additionally, the illogical and irresponsible use of antibacterial medications suppresses susceptible bacterial species, which contributes to the development of resistant strains at the level of the farm (Saeed *et al*., 2023; Thomrongsuwannakij *et al*., 2022; Jhandai *et al*., 2025). The existence of carrier animals that move across animal herds and vector action are two of the many factors that contribute to the spread of pathogens that are resistant to antibiotics. Management interventions, infection control, and vaccinations are the main key points of avian colibacillosis control. A variety of antimicrobial drugs, such as β-lactams (penicillins, cephalosporins), aminoglycosides, tetracycline, sulphonamides, and fluoroquinolones, are used to treat chicken colibacillosis. Antimicrobial resistance against APEC is caused by selection pressure due to the regular use of antimicrobial drugs (Ibrahim *et al*., 2019). Various β-lactam genes were studied in APEC isolates with ESBL genes and ranged from 6.6% to 100%. (Qabajah *et al*., 2014; Li *et al*., 2015; Younis *et al*., 2017; Ibraheem *et al*., 2019; El Seedy *et al*., 2019; Dhaouadi *et al*., 2020; Borges *et al*., 2023; Saeed *et al*., 2023; Pilati *et al*., 2024; Bhattarai *et al*., 2024; Patel *et al*., 2024**;** Müller *et al*., 2024; Tongkamsai and Nakbubpa, 2024; Jhandai *et al*., 2025; Chenouf *et al*., 2025).

Integrons are important mobile genetic factors which help in the spread of resistance. Whereas Class-2 integrons are more uncommon and expanded, Class-1 integrons are more distinct and play an important part in the development of antibiotic resistance. Class-2 integrons were effective in distributing antibiotic resistance genes throughout the environment and encouraging horizontal gene transfer due to several of their appurtenances. Class-3 integrons are uncommon, contributing to the development of multidrug resistance at a lower rate (Zhang *et al*., 2020). Several studies detected the occurrence rate of integrone genes in APEC isolates (Ahmed *et al*.,2013; Oosterik *et al*., 2014; Kilani *et al*., 2015; Cavicchio *et al*., 2015; Awad *et al*., 2016; *Yoon et al., 2020;* Dhaouadi *et al*., 2020; Kang *et al*., 2022; Patel *et al*., 2024; Jhandai *et al*., 2025). These studies showed that the incidence rate for class 1 was between 18%-97%, while for class 2 it was between 0.0%-53.1%.

Up till now, Palestine has limited information about APEC strains. Only 3 reports were published about colibacillosis (Qabajah *et al*., 2014; Thabet *et al*., 2023; Adwan *et al*., 2024). The *blaCTX-M* genes were only detected in one previous study (Qabajah *et al*., 2014). Additionally, ESBL genes are frequently transmitted by plasmids that can be transferred to different *Enterobacteriaceae*; therefore, creating a danger of spreading ESBL and MBL genes to other animals and humans. For this reason, it is essential to detect the dissemination of ESBL- and MBL-producing APEC. This study was carried out to evaluate the prevalence and molecular characterization of ESBLs and MBLs producing APEC isolates using multiplex PCR technique and to assess the prevalence of class 1, 2 and 3 integrons in these isolates. In addition, we sought to evaluate the genetic heterogeneity among APEC isolates using RAPD-PCR technique. There has not been any prior research on this topic in Palestine.

MATERIALS AND METHODS

Sample collection and DNA isolation: A total of 65 deceased chickens assumed of having colibacillosis were brought in for postmortem investigation, and a pool of samples was used to make the diagnosis. Samples of the heart, liver, peritoneum, and lung from broiler farms in northern Palestine formed each pool. These samples were obtained from chickens of different ages that had lesions of omphalitis, cellulitis, salpingitis, egg peritonitis, airsacculitis, perihepatitis, and pericarditis during the postmortem examination. The samples were obtained

under aseptic conditions using a sterile cotton swab in a sterile 5 ml nutrient-rich broth in a laboratory belonging to these at farms. This study was performed in 2024 between May and July. Samples were collected from 16 farms in the Northern Palestine (Adwan *et al*., 2024).

The DNA samples which were used in the previously published study (Adwan *et al*., 2024), were utilized to detect β-lactamases genes, class 1, 2 and 3 integrons and RAPD-PCR.

Detection of ESBL genes: The ESBL gene sequences encoding the *blaTEM*, *blaSHV*, *blaCTX-M*, and *blaOXA* enzymes were detected using multiplex PCR. The oligonucleotide primer sets, expected amplicon sizes (bp) and annealing temperature for these genes are shown in Table 1. Reaction volume and PCR thermal conditions were conducted as described previously (Adwan and Abu Jaber, 2016). In summary, the PCR reaction was performed in 25µL volume, composed of 12.5µL of PCR premix with $MgCl_2$ (GoTaq® Green Master Mix, Promega), 0.75µL of 10µM solution of each primer, 3µL (40-60 ng) of the APEC DNA template, then free DNA nuclease PCR water was added to complete the reaction mixture up to a final volume 25.0µL. The following PCR temperatures and time conditions were used to perform ESBL genes amplification using a thermal cycler (Mastercycler Personal, Eppendorf): 3 minutes at 94°C (initial denaturation); for 25 cycles 30 seconds at 94°C (denaturation), 30 seconds at 60°C (annealing) and 2 minutes at 72°C (extension), then a final step for 5 minutes at 72°C (final extension). The PCR product was subjected to electrophoresis on 1.5% agarose gel to identify the size of the amplified PCR fragment, after staining with 0.5 µg/ml of ethidium bromide dye. The size of amplified fragments was detected using 100-bp DNA ladder (GeneDireX). Positive control strains for different ESBL genes (department collection) were used in this study and PCR reaction without DNA was conducted as negative control.

Detection of MBL genes: The multiplex PCR technique was used to identify MBL gene sequences that code for the *blaVIM*, *blaSPM*-1, and *blaSIM*-1 enzymes. Table 1 lists the primer sequences, the expected amplicon size (bp) and the annealing temperature for these genes. The PCR reactions were conducted and amplified products were detected as well as in detection of ESBL genes. The following PCR temperatures and time conditions were used to perform MBL genes amplification using a thermal cycler (Mastercycler Personal, Eppendorf): 5 minutes at 94°C (initial denaturation); followed by 35 cycles of 30 seconds at 94°C (denaturation), 40 seconds at 52°C (annealing) and 50 seconds at 72°C (extension), then final step for 5 minutes at 72°C (final extension) (Adwan and Rabaya, 2016). Positive control strains for different MBL genes (department collection) were used in this study and PCR reaction without DNA was carried out as negative control.

Detection of class 1, 2 and 3 integrons: All APEC isolates were analyzed for the presence integrase genes *intI1*, *intI2*, and *intI3* using specific primers. Table 1 shows the primer sequences, expected amplified fragment lengths and annealing temperature for these genes. The PCR reactions were conducted and amplified products were detected as in detection of ESBL genes. The following PCR temperatures and time conditions were used to perform integron genes amplification using a thermal cycler (Mastercycler Personal, Eppendorf): 30 cycles of 30 seconds at 94ºC (denaturation), 30 seconds at 58ºC for (annealing) and 60 seconds at 72ºC (extension), then final step for 2 minutes at 72ºC (final extension) (Adwan and Rabaya, 2016). Positive control strains for different integrase genes (department collection) were used in this study and PCR reaction without DNA was performed as negative control.

Random amplification of polymorphic DNA PCR (RAPD-PCR): The RAPD primer 208 was used for RAPD-PCR. Table 1 shows the primer sequence and annealing temperature. Briefly, the PCR reaction was performed in 25µl volume, composed of 12.5µL of PCR premix with $MgCl_2$ (GoTaq® Green Master Mix,

Table 1: Target genes for PCR amplification, amplicon size, primer sequences and annealing temperature used in this study.

Group	Targets	Primer sequence 5′→3′	Amplicon size (bp)	Primer mix	Ann. Temp.	References
Extended spectrum β-lactamases (Class A)	*blaSHV*	SHV F 5-ATG CGT TATATT CGC CTG TG-3 SHV R 5-TGC TTT GTT ATT CGG GCC AA-3	747	I		Adwan and Abu Jaber, 2016
	blaTEM	TEM F 5-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3 TEM R 5-ACG CTC ACC GGC TCC AGA TTT AT-3	445	I		
	blaCTX-M	CTX-M F 5-ATG TGC AGY ACC AGT AAR GTK ATG GC-3 CTX-M R 5-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3	593	I	60°C	
	blaKPC	KPCM F 5-CGTCTAGTTCTGCTGTCTTG-3 KPCM R 5-CTTGTCATCCTTGTTAGGCG-3	789	I		
Extended spectrum β-lactamases (Class D)	*blaOXA*	OXA F 5-ATT ATC TAC AGC AGC GCC AGT G-3 OXA R 5-TGC ATC CAC GTC TTT GGT G-3	296	I		
Metallo-β-lactamases (Class B)	*blaVIM*	Vim-F 5-GATGGTGTTTGGTCGCATA-3 Vim-R 5-CGAATGCGCAGCACCAG-3	390	2		Adwan and Rabaya, 2016
	blaSPM	Spm-F 5-AAAATCTGGGTACGCAAACG-3 Spm-R 5-ACATTATCCGCTGGAACAGG-3	271	2	52°C	
	blaSIM	Sim-F 5-TACAAGGGATTCGGCATCG-3 Sim-R 5-TAATGGCCTGTTCCCATGTG-3	570	2		
Integrons 1, 2 and 3	*intI1*	intI1 F 5-GCATCCTCGGTTTTCTGG-3 intI1 R 5-GGTGTGGCGGGCTTCGTG-3	457	3		Adwan and Rabaya, 2016
	intI2	intI2 F 5-CACGGATATGCGACAAAAAGG T-3 intI2 R 5-GTAGCAAACGAGTGACGAAATG-3	789	3	58°C	
	intI3	intI3 F 5-AT TGCCAAACCTGACTG-3 intI3 R 5-CGAATGCCCCAACAACTC-3	922	3		
RAPD		208 5'-ACG GCC GAC C-3			32°C	Adwan and Omar, 2021

Promega), 2µl of 10µM solution of RAPD PCR primer 208, 3µL (40-60 ng) of the APEC DNA template, then free DNA nuclease PCR water was added to complete the reaction mixture up to a final volume 25.0µL. In addition, the concentration of dNTPs and $MgCl_2$ in master mix was raised by adding 0.5µL of 10 mM dNTPs and 1.5µL of 25 mM $MgCl_2$. The following conditions were used to perform RAPD-PCR: 94℃ for 3 minutes (initial denaturation); followed by 35 cycles: 94℃ for 1 minute (denaturation), 32℃ for 1 minute (annealing) and 72℃ for 2 minutes (extension), then a final step at 72℃ for 5 minutes (final extension) (Adwan and Omar, 2021). The PCR products were detected as well as in case detection of ESBL genes.

The PCR bands on the gels were scored using a binary scoring system, where 0 or 1 denoted the absence or presence of PCR fragments. The weighted pair group method for arithmetic averages (UPGMA) was used to evaluate a binary matrix created using IBM's SPSS statistics software version 20.

RESULTS

Detection of ESBL and MBL genes: According to the results of the current study, the occurrence of extended-spectrum β-lactamase genes was 65 isolates (100%), 29 isolates (44.6%) and 1 isolate (1.6%) for *blaTEM*, *blaCTX-M* and *blaOXA*, respectively. However, both *blaSHV* and *blaKPC* were not detected in these isolates. The occurrence in combination was 53.8%, 44.6% and 1.6% for *blaTEM* alone, *blaTEM* and *blaCTX-M*, and *blaTEM* and *blaOXA*, respectively. The ESBL genes detected in this study (Fig. 1).

In this study, results showed that the occurrence of MBL genes among APEC isolates was 47 (72.3%) isolates, 2 (3.1%) isolates and 0 (0.0%) isolate for *blaSIM*, *blaVIM* and *blaSPM*, respectively. The 2 isolates, which had *blaVIM* genes, co-existed with *blaSIM* genes. The MBL genes detected in this study (Fig. 2).

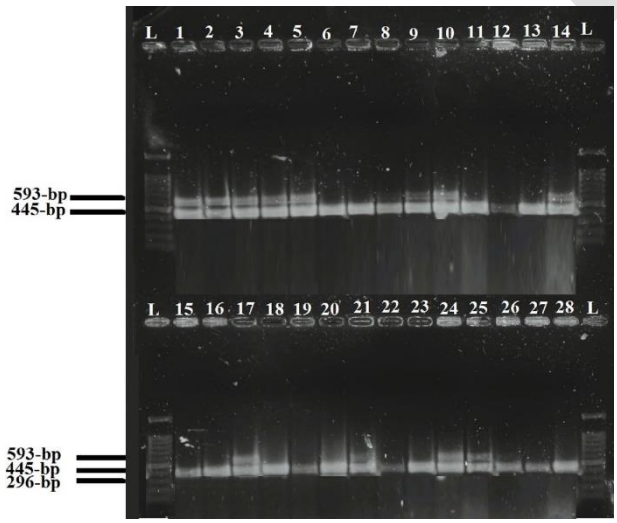

Fig. 1: Multiplex PCR profiles specific for ESβL genes of class (A) and class (D) investigated in APEC isolates. L represents 100-bp ladder, other lanes for detected ESβL genes; *blaOXA* (296-bp), *blaCTX-M* (593-bp) and *blaTEM* (445-bp).

Detection of class 1, 2 and 3 integrons: Results of the current study showed that 65 of APEC isolates (100%) had class 1 integrons and 23 isolates (35.4%) carried class 2 integrons. All Class 3 integrons were not detected in all tested isolates. Results of class 1 and class 2 integrons (Fig. 3).

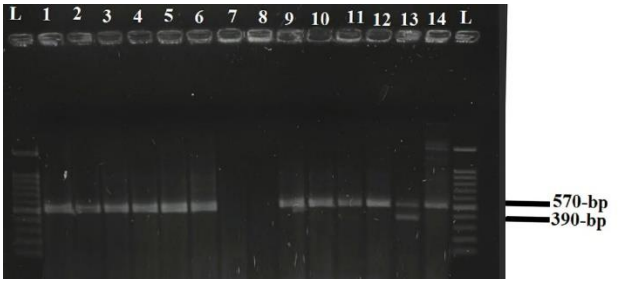

Fig. 2: Multiplex PCR profiles specific for MBL genes of class (B) investigated in APEC isolates. L represents 100-bp ladder, other lanes for detected MBL genes; *blaVIM* (390-bp) and *blaSIM* (570-bp).

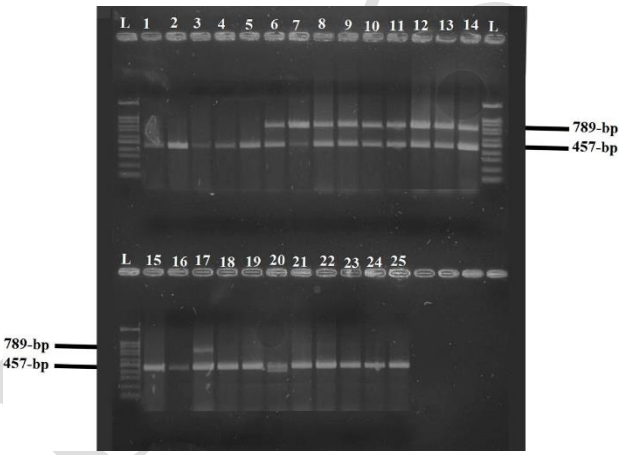

Fig. 3: Multiplex PCR profile for integrons detected in APEC isolates. L represents 100-bp ladder, Lanes 1-25 represent class 1 integron (457-bp) and class 2 (789-bp).

Among the 65 APEC isolates, 11 gene patterns for integrons, ESBLs and MBLS genes were detected. The most common pattern was *intI1*, *blaSIM*, *blaTEM*, *blaCTX-M*, which had an occurrence rate 30.8%. Patterns of these genes (Table 2).

Table 2: Integrons, ESBLs and MBLs genes patterns of 65 APEC isolates collected from broiler farms in northern Palestine

	Pattern	No. of isolates (%)
1	*intI1, blaSIM, blaTEM, blaCTX-M*	20 (30.9)
2	*intI1, blaSIM, blaTEM*	14 (21.5%)
3	*intI1, blaTEM*	6 (9.2%)
4	*intI1, intI2, blaTEM*	8 (12.3%)
5	*intI1, blaVIM, blaSIM, blaTEM*	1 (1.5%)
6	*intI1, intI2, blaVIM, blaSIM, blaTEM*	1 (1.5%)
7	*intI1, intI2, blaTEM, blaOXA*	1 (1.5%)
8	*intI1, intI2, bla SIM, blaTEM, blaCTX-M*	6 (9.2%)
9	*intI1, intI2, blaSIM, blaTEM*	5 (7.7%)
10	*intI1, intI2, blaTEM, blaCTX-M*	2 (3.1%)
11	*intI1, blaTEM, blaCTX-M*	1 (1.5%)

RAPD-PCR analysis: The RAPD-PCR typing of 25 APEC isolates which carried genes for integrons, ESβLs and MBLs were genetically diverse and composed of a heterogeneous population with a total of 8 RAPD-PCR clusters at a 50% similarity level. Cluster C1 and C2 are divided into 6 and 3sub-clusters, respectively. Results of RAPD-PCR clusters and subclusters (Fig. 4).

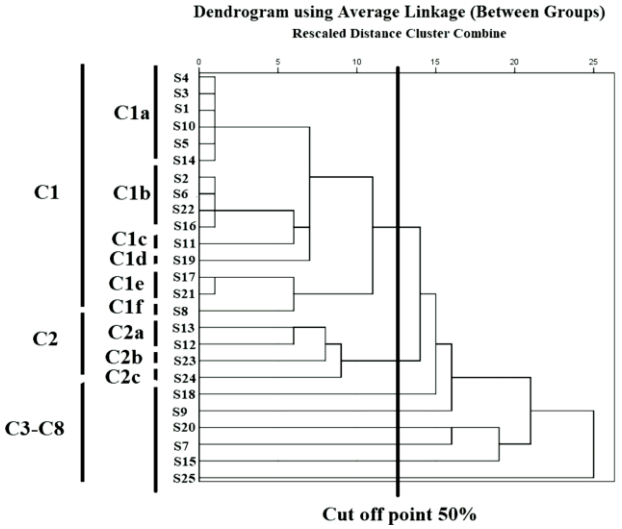

Fig. 4: Dendrogram of 25 APEC *isolates* carried genes for ESβLs and, MBLs and integron genes based on the UPGMA method derived from analysis of the RAPD-PCR-profiles at a 50% similarity level. C: Cluster.

DISCUSSION

Antibiotic resistance has become an increasing threat to public health worldwide. In addition to establishing drug-resistant pathogenic bacteria, antibiotic misuse or abuse changes the selective pressure on beneficial microbes. Antibiotics are utilized frequently in animal husbandry all around the world to promote growth. This practice has led to the development of antibiotic-resistant microbes. Because *E. coli* survives as a normal microbiota in both animal and human, investigating the prevalence of antibiotic resistance in this microorganism from various sources may show situations of either the vertical gene transfer of bacterial clonality or horizontal transfer of genes among various types of bacteria. Furthermore, *E. coli* has been shown to be an exceptionally effective source of antibiotic resistance genes. Multi-drug-resistant strains of *E. coli* can be attributed to accumulation of resistance genes using genetic mobile elements such as transposons, plasmids and integrons. Resistance genes may develop by two ways: either the acquisition of resistant genes from other bacteria belonging to the same or other species or alteration at gene-level (Husna *et al*., 2023).

Antimicrobial drugs, such as penicillins, aminoglycosides, quinolones, fluoroquinolones, folate pathway inhibitors, and β-lactams are used to treat avian colibacillosis infections (Yoon and Lee, 2022; Misumi *et al*., 2023). β-lactam antimicrobial agents are widely employed to treat bacterial infections in both humans and animals, leading to the emergence of ESBL-producing APEC worldwide. ESBL-producing *E. coli* isolates recovered from food animals are considered a public health problem (Yoon and Lee, 2022). Thus, emergence of β-lactamase-encoding genes in broiler farms may influence the dissemination of the gene among bacterial populations by plasmid-mediated horizontal gene transfer. Therefore, tracking is required to follow the movement of β-lactamase-encoding genes between different reservoirs in the chicken industry (Kang *et al*., 2022).

Integrons are significant mobile genetic components which assist in resistance transmission. Because of its recombination abilities, Class 1 is the most frequently detected integron identified in clinical isolates of Gram-negative bacteria such as *E. coli* and bacteria that are multidrug-resistant (Kaushik *et al*., 2018). Class 1 and 2 integrons are frequently associated with antibiotic resistance gene cassettes in isolated bacteria, particularly those of the *Enterobacteriaceae* family (Kang *et al*., 2022). The APEC isolates with class 1 or 2 or both integrons possessed gene cassettes linked to drug resistance, which revealed elevated resistance rates, imparted resistance to multiple antibiotic classes, and greater potential for horizontal transmission (Cavicchio *et al*., 2015; Kang *et al*., 2022). This result contrasted with other previous studies which showed that the occurrence rate of *intI1* gene is lower than the results obtained in our study. The positivity was 46.6% and 25.9% in Egypt (Ahmed *et al*., 2013; Awad *et al*., 2016), 21.6% in Belgium (Oosterik *et al*., 2014), 49.8% in Italy (Cavicchio *et al*., 2015), 24.2 and 32.7% in South Korea (Yoon *et al.,* 2020; Kang *et al*., 2022), 18% in Tunisia (Dhaouadi *et al*., 2020), 46.4 and 68% in India (Patel *et al*., 2024; Jhandai *et al*., 2025), respectively. Similar results were obtained in Jordan (Ibraheem *et al*., 2019), where positivity of 97% was documented.

The occurrence rate of *intI2* gene (35.4%) in the current study was lower or higher than previous studies reported from different geographical regions. The positivity was 9.6 and 3.4% in Egypt (Ahmed *et al*., 2013; Awad *et al*., 2016), 0.0% in Belgium (Oosterik *et al*., 2014), 10.4% in Italy (Cavicchio *et al*., 2015), 3 and 53.1% in South Korea (Yoon *et al.,* 2020; Kang *et al*., 2022). The *intI3* gene sequence was not detected in all our isolates, this result is consistent with previous studies (Oosterik *et al*., 2014; Cavicchio *et al*., 2015; Awad *et al*., 2016). Results of the current study showed that class 1 was the most prevalent among APEC isolates in Palestine, however, these results were inconsistent with previous studies conducted in Tunisia (Kilani *et al*., 2015) and South Korea (Kang *et al*., 2022), which showed that class 2 was the most prevalent among APEC isolates. In this study, 100% of APEC isolates carried integron genes. These results were incompatible with previous studies that reported a total of 55% (Cavicchio *et al*., 2015) and 29.3% (Awad *et al*., 2016) of APEC isolates harbored integron gene sequences. Integron gene frequency is affected by many factors such as the number of samples tested, type of tested class and the primer specificity.

Results of this report showed that 100% of APEC isolates carried ESBL genes. These results were in agreement with studies previously published in Egypt (El Seedy *et al*., 2019), which showed that 100 % of the tested APEC isolates harbored ESBL genes. However, results of this study were higher than other studies carried out in several regions. The incidence rate was 12.1% in Palestine (Qabajah *et al*., 2014), 75.9% in China (Li *et al*., 2015), 91.8% in Egypt (Younis *et al*., 2017), 72.9% in Jordan (Ibraheem *et al*., 2019), 30% in Tunisia (Dhaouadi *et al*., 2020), 6.6% and 64.1% in Brazil (Borges *et al*., 2023; Pilati *et al*., 2024), 45.1% in Pakistan (Saeed *et al*., 2023), 33.7% in Nepal (Bhattarai *et al*., 2024), 85.5 and 62% in India (Patel *et al*., 2024; Jhandai *et al*., 2025**),** 52.3% in Germany (Müller *et al*., 2024), 81.8% in Thailand (Tongkamsai and Nakbubpa, 2024), 8% in Algeria (Chenouf *et al*., 2025). APEC isolates had ESBL genes. Some of these studies detected one gene. The variation in prevalence of ESBL-

positive isolates across studies may be caused by differences in the intensive and unacceptable use of antibiotics, variations in commonly used antimicrobial agents, means of detection such as molecular or conventional methods, and the specificity of primers in molecular techniques. Closely placed farms were important risk variables linked to the existence of MDR APEC in broiler chickens, and other factors including infection control measures, illness prevention, and the use of polluted ground water also affected the incidence of ESBL.

This investigation demonstrated a significant frequency of ESβL-producing isolates in broilers. This reflects an important threat to public health because of these bacterias' ability to break down third-generation cephalosporins, which are usually used to treat severe infections, as well as the possibility of the dissemination and transmission of the resistant genes to human population through the food chain, direct contact, or the environment (Awad *et al*., 2016).

According to the results of the current study, the occurrence of ESBL genes was 65 isolates (100%), 29 isolates (44.6%) and 1 isolate (1.6%) for *blaTEM*, *blaCTX-M* and *blaOXA*, respectively. However, both *blaSHV* and *blaKPC* were not detected in these isolates.

Results of this report showed that 100% of Palestinian APEC isolates carried *blaTEM* genes. These results were consistent with a report previously published in Egypt (El Seedy *et al*., 2019), where it showed that 100% of the tested APEC isolates harbored *blaTEM* genes. Results of other previous studies showed lower occurrence rates such as 16.1% in China (Li *et al*., 2015), 78% Egypt (Younis *et al*., 2017), 72.9% in Jordan (Ibrahim *et al*., 2019), 12.0% in Tunisia (Dhaouadi *et al*., 2020), 29.1% and 32.7% in South Korea (Kim *et al*., 2020; Kang *et al*., 2022), 36.4 and 81.8% in Thailand (Thomrongsuwannakij *et al*., 2022; Tongkamsai and Nakbubpa, 2024), 45.9% in Pakistan (Saeed *et al*., 2023), 69.6 and 68% in India (Patel *et al*., 2024; Jhandai *et al*., 2025), 43.3% in Brazil (Pilati *et al*., 2024), 33.7% in Nipal (Bhattarai *et al*., 2024), 52.3% in Germany (Müller *et al*., 2024) and 2.4% in Algeria (Chenouf *et al*., 2025)**.** The high prevalence of plasmid-mediated beta-lactamases, *blaTEM* genes among APEC isolates in the current research study, is a concerning finding because these genes are plasmid-borne and may disseminate to other pathogenic microorganisms, rendering the most recent generation cephalosporin useless and ineffective (Bhattarai *et al*., 2024). In addition, the *blaCTX-M* genes were detected in several geographical areas and these results were similar or different from results obtained in this study. The occurrence rate of *blaCTX-M* genes was 12.1% in Palestine (Qabajah *et al*., 2014), 70.1% in China (Li *et al*., 2015), 35.3% in Egypt (Awad *et al*., 2016), 28% in Tunisia (Dhaouadi *et al*., 2020), 8.9% and 49.0% in South Korea (Kim *et al*., 2020; Kang *et al*., 2022), 26.3% in Japan (Misumi *et al*., 2023), 87.8 in Pakistan (Saeed *et al*., 2023), 13% in India (Patel *et al*., 2024), 81.8% in Thailand (Tongkamsai and Nakbubpa, 2024), and 7.6 % in Algeria (Chenouf *et al.*, 2025). ESBLs, including *blaCTX-M*, which hydrolyze the β-lactam ring, are major issues as they confer resistance to the nearly all β-lactam antibiotics, including cephalosporins (Kim *et al*., 2020). The *blaCTX-M* gene is one of the most common β-lactamase genes and ESBL-producing *E. coli* strains carrying the *blaCTX-M* gene that have been reported not only in food animals like chickens but also in humans (Yoon and Lee, 2022). In particular, the CTX-M-1 and CTX-M-55 genes are common genotypes in human patients from different Asian countries (Zhang *et al*., 2014; Misumi *et al*., 2023). The occurrence rate of *blaOXA* genes according to the geographical area was 25.3% in China (Li *et al*., 2015), 12.1% in Egypt (Awad *et al*., 2016), 2.9% in India (Patel *et al*., 2024), 0.0% in Thailand (Tongkamsai and Nakbubpa, 2024). In addition, the incidence of *blaSHV* was 0.0% in China (Li *et al*., 2015), 0.0% and 23.3% in Egypt (Awad *et al*., 2016; Younis *et al*., 2017), 1.8% in Jordan (Ibrahim *et al*., 2019), 4% in Tunisia (Dhaouadi *et al*., 2020), 3.0% and 0.0% in Thailand (Thomrongsuwannakij *et al*., 2022; Tongkamsai and Nakbubpa, 2024), 2.9% in India (Patel *et al*., 2024), 1.6% in Brazil (Pilati *et al*., 2024), and 0.5% in Algeria (Chenouf *et al*., 2025). Third and fourth cephalosporin generations are rarely used in Palestinian poultry farms due to their expensive prices. However, Ceftriaxone and Ceftiofur, which belonged to the third generation of cephalosporins, exhibited high resistance 89.2 and 92.3% against these APEC isolates in Palestine, respectively (Adwan *et al*., 2024). Thus, emergence of *blaCTX-M* genes in broiler farms in Palestine may influence the spreading and transmission of these genes among bacterial populations by plasmid-mediated horizontal gene transfer or emergence of third and fourth generations of cephalosporins resistant isolates may be independent of the use of these antibiotics in farms. The use of other antibiotics that have β-lactam rings, such as penicillin and penicillin derivatives, may induce selective resistance toward third and fourth generation of cephalosporins and lead gene expression of β-lactamase genes. The products of *blaCTX-M* genes are responsible for the breakdown of β-lactam rings in the third and fourth generation of cephalosporins, according to penicillin and penicillin derivatives that are commonly used in poultry farms (Kang *et al*., 2022; Yoon *et al*., 2020). Amoxicillin is one of the penicillin derivatives commonly used in poultry farms in Palestine, the APC isolates showed complete resistance toward amoxicillin (Adwan *et al*., 2024), the products of *blaTEM* genes are responsible for the inactivation of β-lactam ring in penicillin and penicillin derivatives including amoxicillin. High resistance to ciprofloxacin (86.2%), norfloxacin (89.2%) and enrofloxacin (98.5%) (Adwan et al., 2024), is due to plasmid-mediated quinolone resistance genes. The presence of the plasmid-mediated quinolone resistance genes may be significantly associated with the β-lactamase genes, perhaps due to common carriage on a plasmid in *Enterobacteriaceae* (Yoon *et al*., 2020). Therefore, monitoring ESBL genes is critical to track the dissemination of these genes between different reservoirs in the poultry industry.

In this research, results showed that the occurrence of MBL genes among APEC isolates was 47 (72.3%) isolates, 2 (3.1%) isolates and 0 (0.0%) isolates for *blaSIM*, *blaVIM* and *blaSPM*, respectively. The widespread introduction of MBL-encoding genes throughout pathogenic bacteria such as *E. coli* is currently an important factor contributing to antibiotic resistance. The MBL gene *blaSIM* first emerged in *Acinetobacter baumannii* (Lee *et al.,* 2005). Our research results demonstrated the emergence of 47 blaSIM-2-

producing APEC isolates among a total of 65 isolates, with a 72.3% incidence. Additional studies must be conducted to find possible causes for the spread of the *blaSIM* gene to APEC isolates obtained from broiler farms in Northern Palestine. In the current research study, approximately 78.5% of APEC isolates had 2 or more than 2 β-lactamase genes. The occurrence of these combinations was 40, 29.2, 4.6, 3.1 and 1.5% for *blaSIM-blaTEM-blaCTXM*, bla*SIM-blaTEM*, *blaTEM-blaCTXM*, bla*VIM-blaSIM-blaTEM* and *blaTEM-blaOXA*, respectively. Coexistence of various β-lactamase genes were detected among the APEC isolates in previously published studies (Dhaouadi *et al*., 2020; Saeed *et al*., 2023; Pilati *et al*., 2024). The coexistence of distinct β-lactamase genes is correlated with raised resistance (Gundran *et al*., 2019).

This study revealed that the 65 APEC isolates demonstrated 11 gene patterns for integrons, ESBLs, and MBLs genes. Furthermore, RAPD-PCR typing of 25 APEC isolates indicated 8 RAPD-PCR clusters with a 50% similarity level. Each cluster has identical APEC isolates that have the same band patterns or highly similar band patterns. Cluster C1 and C2 are separated into 6 and 3 sub-clusters, respectively. The results obtained showed that APEC isolates were highly genetically varied and belonged of a heterogeneous population. The results presented coincided with a recent published study using the same isolates (Adwan *et al*., 2024), that demonstrated notable heterogenic differences according to antibiotic resistance and virulence factor patterns. It showed that these 65 APEC isolates had 34 virulence gene patterns and 48 resistance patterns and these resistance patterns formed 7 cluster groups (C1-C7) during a clustering process to form a dendrogram. In addition, clusters C-1 to C4 can be separated into other sub-clusters. All of these results indicate that the APEC isolates obtained from broiler farms in Northern Palestine have a notable genetic diversity.

Conclusions: This study revealed an elevated rate of potentially ESBL- and MBL-producing APEC isolates, indicating that broiler chickens in Palestine might serve as a reservoir for these types of genes which could represent a public health danger. Intensive monitoring, improved management protocols and continuous surveillance are required in broiler farms in Northern Palestine to recognize critical control points that can prevent the rapid development horizontal transmission of β-lactamases-resistant APEC.

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