Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.016

RESEARCH ARTICLE

Antibiotic Resistance Pattern of Extraintestinal Pathogenic *E. coli* Isolates Obtained from Broiler Flocks in Northern Palestine

Sameh Abuseir[1]*, Ghadeer Omar[2]*, Mahmoud Albzour[3] and Ghaleb Adwan[2]

[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine and Agricultural Engineering, An-Najah National University, Nablus, Palestine; [2]Department of Biology and Biotechnology, Faculty of Science, An-Najah National University, Nablus, Palestine; [3] Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.
*Corresponding author: sameh.abuseir@najah.edu; ghaderomar@najah.edu

ARTICLE HISTORY (25-943)

Received:	October 06, 2025
Revised:	January 16, 2026
Accepted:	January 20, 2026
Published online:	February 25, 2026

Key words:
Antibiotic resistant
ExPEC
MARI
Multi-drug resistant
Palestine

ABSTRACT

Extra-intestinal pathogenic *E. coli* (ExPEC) is a causative agent of colibacillosis in poultry, leading to significant economic losses worldwide. This study aimed to evaluate the antimicrobial resistance profile of 325 isolates recovered from deceased broiler chickens with clinical cases of colibacillosis, on different farms in the northern area of the Palestinian territories. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method with 17 commonly used antibiotics. All tested ExPEC isolates showed multidrug resistance (MDR). The highest levels of resistance were seen against amoxicillin (100%), ceftiofur (99.4%), enrofloxacin (99.1%), ceftriaxone (95.4%), levofloxacin (94.2%), gentamicin (92%), norfloxacin (90.5%), and florfenicol (90.2%). Additionally, high resistance levels were observed against ciprofloxacin (84.9%), sulfamethoxazole/trimethoprim (80%), and cephalexin (67.5%). Intermediate resistance was noted for neomycin (45.3%), doxycycline (43.1%), and fosfomycin (40.3%), while low resistance was found for polymyxin B (22.1%), spectinomycin (26.8%), and colistin (10.5%). According to the Multiple Antibiotic Resistance Index (MARI), Palestinian ExPEC isolates are considered high-risk due to their exposure to a high level of antibiotics. Statistical analysis revealed significant differences between various farms. The indiscriminate and excessive use of antibiotics can lead to the emergence of drug-resistant bacterial strains. Alternative methods such as immunization have become more popular to manage ExPEC without relying solely on antibiotics. Understanding antibiotic resistance in ExPEC is crucial for developing effective treatment and control strategies. A comprehensive approach is necessary to prevent and manage avian colibacillosis in Palestine.

To Cite This Article: Abuseir S, Omar G, Albzour M and Adwan G, 2026. Antibiotic resistance pattern of extraintestinal pathogenic *E. coli* isolates obtained from broiler flocks in northern Palestine. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2026.016

INTRODUCTION

Extraintestinal pathogenic *E. coli* (ExPEC) pathotype is responsible for colibacillosis in poultry, leading to significant economic losses globally. It is similar to human pathogenic neonatal meningitis-associated *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC). Treating illnesses caused by newly discovered multidrug-resistant ExPEC strains is becoming increasingly challenging. The potential risks of antibiotic resistance, the genetic connection between human ExPEC and poultry ExPEC, and certain clinical cases in humans with potential animal or foodborne origins have been suggested (Saidenberg *et al.*, 2024). Various strategies may be necessary to manage ExPEC infections. However, as disease incidence increases due to reduced antibiotic use, improved immunizations, or other factors, additional measures may be required (Watts and Wigley, 2024).

Avian colibacillosis infections are typically treated with antimicrobial medications, including β-lactams, quinolones, fluoroquinolones, penicillins, aminoglycosides, and folate pathway inhibitors (Yoon and Lee, 2022; Misumi *et al.*, 2023). Some of these antimicrobial agents, such as penicillin, chloretetracycline, bacitracin, salinomycin, and colistin, are used by farmers for various reasons, such as disease prevention and growth promotion (Azam *et al.*, 2019). The emergence of multidrug-resistant (MDR) and resistant ExPEC isolates has significant implications for colibacillosis treatment (Amer *et al*., 2018; Afayibo *et al*., 2022; Jalil *et al*., 2023; Bhattarai *et al*., 2024; Dong *et al*., 2025). Overuse of antibiotics in poultry has led to antibiotic-resistant strains and the presence of antibiotic residues in poultry products and meat. Controlling ExPEC infections in poultry may be challenging due to increasing antibiotic resistance, especially to important therapeutics like colistin, carbapenems, fluoroquinolones, and β-lactams (Jalil *et al*., 2023). Bacterial antibiotic resistance is a significant concern in animal production, as agricultural operations can be hotspots for the development and spread of antibiotic-resistant infections. It is widely recognized that antibiotic-resistant bacteria can spread from animals to humans through direct contact, manure, food production processes, and vice versa. The use of natural organic fertilizers and agricultural waste can also contribute to the spread of these microbes in the environment (Karpov *et al*., 2024).

In Palestine, there is limited knowledge on the patterns of antibiotic susceptibility among ExPEC isolates recovered from poultry flocks in Palestine. To address this knowledge gap, a study was conducted on various broiler farms in North Palestine.

MATERIALS AND METHODS

Ethical approval and informed consent: Ethics authorization is not essential to such types of work. However, the animals were handled with care while the specimens were being obtained. The animal farm's owner has given their prior permission.

Sample collection: A total of 325 deceased hens suspected of having colibacillosis were brought in for postmortem investigation from 25 farms in Northern Palestine during January 2023 to June 2025. A pool of samples from different parts of the carcass was used to determine the diagnosis. These specimens were collected during the autopsy evaluation of hens with airsacculitis, pericarditis lesions, perihepatitis, omphalitis, egg peritonitis, cellulitis, and salpingitis.

The samples were obtained under aseptic conditions in the laboratory of the farm using a sterile swab in 5 mL tryptone soy broth (TSB: HiMedia Laboratories). The allocation of the isolates according to the farms is presented in Table 1.

Phenotypic E. coli identification: Following a 6-hour incubation period at 37°C, pre-enrichment samples in TSB were subcultured onto eosin methylene blue agar (EMB agar: HiMedia Laboratories). For optimal bacterial growth, the plates were kept for 18 to 24 hours at 37°C. Colonies exhibiting a green metallic sheen were picked for further identification using biochemical testing. The morphological and staining characteristics of the bacteria were examined using Gram staining, which also provided details about potential bacterial identification. It was determined which isolated organisms have growth-supporting traits. The successful identification of the specific *E. coli* bacteria was verified by a group of biochemical tests. The positive samples were further verified by using particular PCR primers.

Table 1: Allocation of 325 ExPEC isolates according to the farms in North Palestine

Farm	No. of isolates (%)
A	14 (4.3)
B	18 (5.5)
C	13 (4.0)
D	9 (2.8)
E	11 (3.4)
F	7 (2.2)
G	12 (3.7)
H	10 (3.1)
I	16 (4.9)
J	12 (3.7)
K	7 (2.2)
L	14 (4.3)
M	9 (2.8)
N	19 (5.8)
O	13 (4.0)
P	18 (5.5)
Q	8 (2.4)
R	8 (2.4)
S	9 (2.8)
T	8 (2.4)
U	11 (3.4)
V	12 (3.7)
W	22 (6.8)
X	25 (7.7)
Y	20 (6.2)
Total	325(100)

DNA extraction: The ExPEC genome was obtained from few colonies cultured on Mueller-Hinton Agar (HiMedia Laboratories), following a previously reported method (Adwan *et al*., 2013). A nanodrop spectrophotometer (GenovaNano, Jenway) was used to analyze the purity and concentration of the extracted DNA. The DNA samples were frozen at -20°C for further examination.

Confirmation of E. coli isolates using PCR technique: For the verification of *E. coli* isolates, the amplification gene *phoA* was employed as the target gene. The sequences of the forward and reverse primer were phoAF: 5-TAC AGG TGA CTG CGG GCT TAT C-3 and phoAR: 5-CTT ACC GGG CAA TAC ACT CAC TA-3, respectively, with a fragment length of 622bp (Adwan *et al*., 2024). The master reaction mixture (GoTaq® Green Master Mix, Promega) was used. The PCR time and temperature settings for DNA amplification, and the gel electrophoresis conditions, were carried out as previously described (Adwan *et al*., 2024). The length of the PCR product fragment, was determined using a 100bp DNA ladder (GeneDireX). In this study, a reference *E. coli* strain (ATCC 25922) was used as a reference positive control.

Antimicrobial sensitivity testing: Antimicrobial sensitivity was determined using the Kirby-Bauer disk diffusion susceptibility test according to the Clinical and Laboratory Standard Institute (CLSI, 2023). All ExPEC samples were assessed for resistance to 17 antibiotics (Oxoid, England) belonged to 10 distinct classes: tetracyclines (doxycycline (DO, 10µg)), cephalosporines (ceftriaxone (CRO, 30µg), cephalexin (CL, 30µg), ceftiofur (FUR, 30µg)), aminopenicillin (amoxicillin (AX, 30µg)), aminoglycosides (gentamicin (CN, 10µg), neomycin (N, 30µg)), fluoroquinolones (ciprofloxacin (CIP, 10µg), norfloxacin (NOR, 10µg), levofloxacin (LEV, 5µg), enrofloxacin (ENR, 5µg)), polymyxins (polymyxin B (PB, 300U), polymyxins E (colistin (CT, 10µg)), amphenicols (florfenicol (FFC, 30µg)), sulfonamides (trimethoprim/sulphamethoxazole (STX, 25µg)), phosphonic antibiotic (fosfomycin (FO, 200µg) and aminocyclitol (spectinomycin (SH, 100µg)). The ExPEC isolates were cultured for 6 to 8 hours and then swabbed onto Mueller-Hinton agar (HMA) plates. Different antibiotic disks were subsequently added to the plates that had been swabbed with *E. coli*. The plates were then incubated at 37°C for 24 hours. The inhibition zones (if any) were identified in accordance with the CLSI protocol (CLSI, 2023). The isolates were categorized into three different groups: resistant, intermediate, and sensitive. A quality control strain of *E. coli* (ATCC 25922) was used as a reference strain in all evaluations of the antimicrobial sensitivity tests.

Determination of multiple antibiotic resistance index (MARI): By calculating their MARI of the ExPEC isolates, the antibiotic resistance profiles were evaluated. The formula MARI = A/B was used to determine the MARI, where A represents the number of antimicrobial resistances of the tested ExPEC isolate and B is the total number of antibiotics for which the isolate was analyzed. A MARI of 0.20 was used to distinguish between low- and high-risk contaminations. When calculating the index for a sample with multiple isolates, the formula A/(B * C) is used, where A is the total antimicrobial resistance score of all isolates in the sample, B is the number of antimicrobial agents analyzed for each isolate, and C is the number of isolates in the sample (Krumperman, 1983). In this study, the MARI was utilized to assess the level of multidrug resistance among farms.

Statistical analysis: The MARI data were analyzed utilizing GraphPad Prism (version 10.4.2; GraphPad Software, San Diego, CA, USA). Due to the data's failure to satisfy normality assumptions, differences among the 25 farms were evaluated using the non-parametric Kruskal–Wallis test. The Kruskal–Wallis statistic was 55.89, with an approximate P value of <0.0002, signifying a statistically significant difference in medians among the groups by applying Dunn's multiple comparisons test (P<0.05 was deemed significant).

RESULTS

A differential diagnosis was conducted using standard conventional microbiological techniques and PCR to identify and confirm and the causal bacterial agent. Features that distinguish the isolated bacterial colonies cultivated on EMB medium which include being Gram-stain negative, short rods, short chains, pairs, or singles, and having a green metallic sheen. Other biochemical tests were also done. Gel electrophoresis indicated that the PCR findings of the *E. coli*-specific *phoA* gene of the 325 bacterial strains showed a PCR amplification of 622-bp band. Table 1 summarizes the relative distribution of these 325 ExPEC isolates from Northern Palestine regions according to their isolation farms, and Fig. 1 shows the amplified amplicon product of the *phoA* gene with 622 bp.

In vitro antibiotic sensitivity pattern of the ExPEC isolates: The tested ExPEC isolates showed a highest degree of resistance toward amoxicillin (100%), ceftiofur (99.4%), enrofloxacin (99.1%), ceftriaxone (95.4%), levofloxacin (94.2%), gentamicin (92%), norfloxacin (90.5%) and florfenicol (90.2%). In addition, a high level of resistance was observed against ciprofloxacin (84.9%), sulfamethoxazole/trimethoprim (80%), and cephalexin (67.5%). Intermediate resistance degree was demonstrated against neomycin (45.3%), doxycycline (43.1%), and fosfomycin (40.3%). However, low resistance degree was detected toward polymyxin B (22.1%), spectinomycin (26.8%), and colistin (10.5%). Phenotypic antibiotic resistance data of the tested 325 ExPEC isolates toward 17 antibiotics are demonstrated in Table 2.

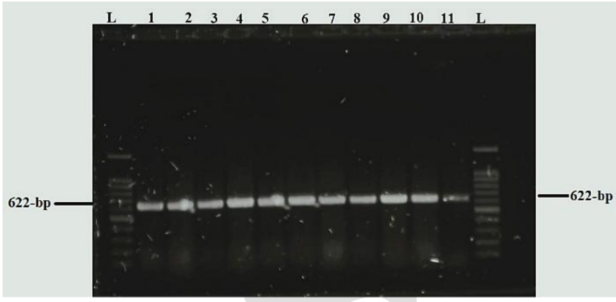

Fig. 1: *Escherichia coli*-specific *phoA* PCR product. Lanes 1-11 are the positive PCR-results of 622-bp fragment, while the L-lane is a 100-bp ladder.

Table 2: The antibiotic sensitivity profile of the ExPEC isolates.

Group	Antibiotic	R	S	I
		Samples (%)	Samples (%)	Samples (%)
Tetracyclines	Doxycycline	140 (43.1)	150 (46.1)	35 (10.8)
Fluoroquinolone	Ciprofloxacin	276 (84.9)	35 (10.8)	14 (4.3)
	Norfloxacin	294 (90.5)	26 (8.0)	5 (1.5)
	Enrofloxacin	322 (99.1)	3 (0.9)	0 (0.0)
	levofloxacin	306 (94.2)	15 (4.6)	4 (1.2)
Aminoglycoside	Gentamicin	302 (92.9)	23 (7.1)	0 (0.0)
	Neomycin	147 (45.3)	172 (52.9)	6 (1.8)
Sulfonamides	Sulfamethoxazole /trimethoprim	260 (80)	62 (19.1)	3 (0.9)
Cephalosporins	Cephalexin	219 (67.4)	105 (32.3)	1 (0.3)
	Ceftriaxone	310 (95.4)	14 (4.3)	1 (0.3)
	Ceftiofur	323 (99.4)	2 (0.6)	0 (0.0)
Aminopenicillin	Amoxicillin	325 (100)	0 (0.0)	0 (0.0)
Polymyxins	Polymyxins E (Colistin)	34 (10.5)	288 (88.6)	3 (0.9)
	Polymyxin B	72 (22.1)	243 (74.8)	10 (3.1)
Amphenicols	Florfenicol	293 (90.2)	29 (8.9)	3 (0.9)
Phosphonic Antibiotic	Fosfomycin	131 (40.3)	191 (58.8)	3 (0.9)
Aminocyclitol	Spectinomycin	87 (26.8)	203 (62.4)	35 (10.8)

In addition, all ExPEC isolates were multidrug-resistant and showed resistance toward more than two classes of antibiotics. All ExPEC isolates demonstrated 188 antibiotic-resistant patterns. The predominant pattern was DO, FFC, CL, CN, FUR, AX, LEV, CIP, CRO, STX, ENR, NOR, with an incidence rate of 4.3%. (Data are not shown).

To see if the isolates' farm had any influence on their level of resistance, their MARI was compared. The MARI of ExPEC isolates recovered from farm K (0.56±0.06) was the lowest compared to the ExPEC isolates recovered from farm I (0.77±0.08) and farm J (0.76 ±0.13). The mean difference of the MARI (0.21) demonstrated that the farm I and farm J ExPEC isolates were resistant to 4 additional antibiotics as compared to the farm K ExPEC isolates on average. The maximum value of the MARI for the isolate was found to be 1.0 in farm J, while the lowest MARI was found to be 0.29 in farm B ExPEC isolates. In this study, all of the ExPEC isolates exhibited MARI ≥ 0.29, and had a range from 0.29 to 1.0, suggesting that these ExPEC isolates showed significant resistance toward antibiotics in these isolates. Dunn's multiple comparisons test statistical analysis showed a significant difference at $P < 0.05$ between farm A vs. I (P=0.0136), farm I vs. K (P=0.0008), farm J vs. K (P=0.0107), and farm K vs. W (P=0.0131). Data are shown in Table 3, Fig. 2, 3, and 4. Generally, the major number of isolates resistant to 11 and 12 antimicrobials with a MARI of 0.65 (26.15%, n = 85) and 0.71 (26.15%, n = 85), respectively, followed by 13 antimicrobials with a MARI of 0.76 (16.00%, n = 52). Table 4 shows data regarding the antimicrobial resistance profiles of ExPEC isolates using the MARI.

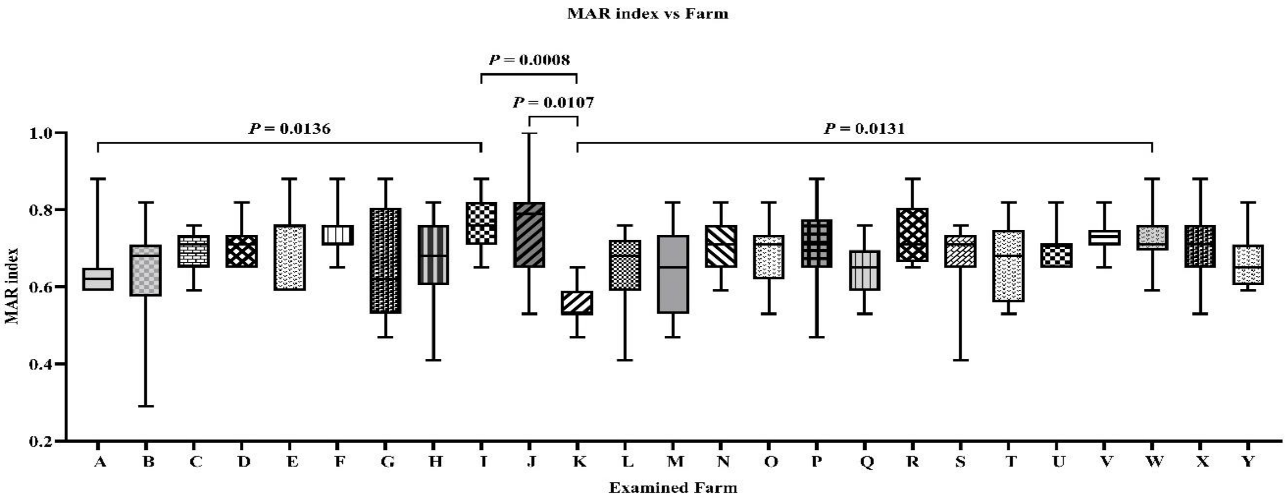

Fig. 2: A box plot diagram demonstrating the comparison the MARI of 25 poultry farms from which the ExPEC strains were isolated; the means were usually different for each site, and statistically significant differences between farms are indicated on the diagram.

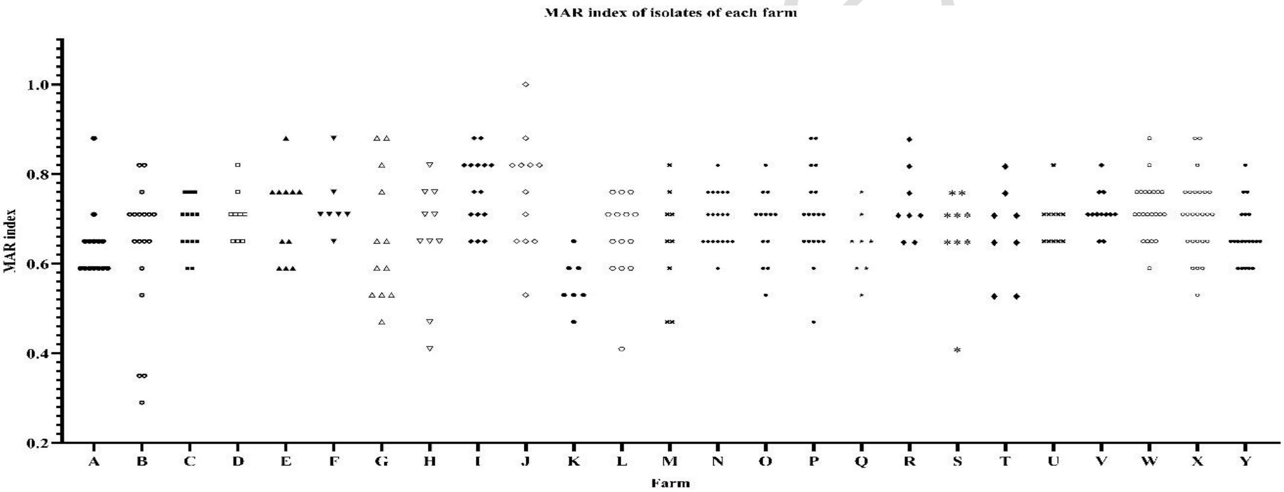

Fig. 3: A scatter plot diagram demonstrating the MARI of ExPEC isolates of each farm; each dot indicates MARI for each isolate to illustrate data variability and distribution.

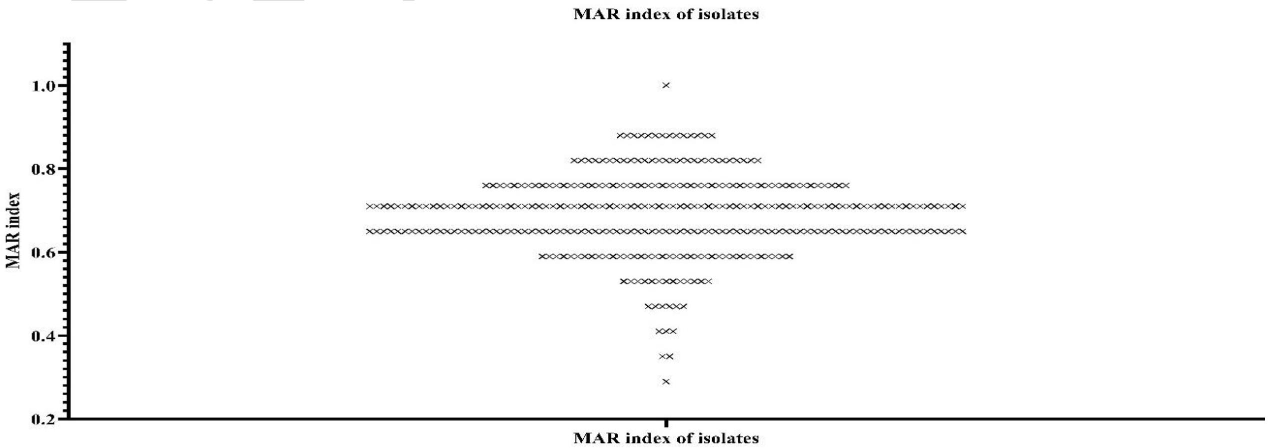

Fig. 4: A scatter plot diagram demonstrating the MARI of ExPEC isolates; each dot indicates MARI for each isolate to illustrate data variability and distribution.

Table 3: Distribution of 325 ExPEC isolates according to the farms, aggregate antibiotic resistance score for each farm, range of MARI, and MARI of ExPEC isolates collected from broiler flocks between 2023 and 2025 in North of Palestine.

Farm	No. of isolates (%)	aggregate antibiotic resistance score of the farm	*Range of MARI	**MARI of the farm ±SD
A	14 (4.3)	152	0.59-0.88	0.64±0.08
B	18 (5.5)	193	0.29-0.82	0.63±0.16
C	13 (4.0)	151	0.59-0.76	0.68±0.06
D	9 (2.8)	108	0.65-0.82	0.71±0.06
E	11 (3.4)	132	0.59-0.88	0.7±0.1
F	7 (2.2)	87	0.65-0.88	0.73±0.07
G	12 (3.7)	134	0.47-0.88	0.66±0.14
H	10 (3.1)	112	0.41-0.82	0.66±0.13
I	16 (4.9)	209	0.65-0.88	0.77±0.08
J	12 (3.7)	155	0.53-1.0	0.76±0.13
K	7 (2.2)	66	0.47-0.65	0.56±0.06
L	14 (4.3)	157	0.41-0.76	0.66±0.1
M	9 (2.8)	99	0.47-0.82	0.65±0.12
N	19 (5.8)	226	0.59-0.82	0.7±0.06
O	13 (4.0)	151	0.53-0.82	0.68±0.08
P	18 (5.5)	217	0.47-0.88	0.71±0.10
Q	8 (2.4)	87	0.53-0.76	0.64±0.07
R	8 (2.4)	101	0.65-0.88	0.74±0.08
S	9 (2.8)	102	0.41-0.76	0.67±0.11
T	8 (2.4)	91	0.53-0.82	0.67±0.1
U	11 (3.4)	129	0.65-0.82	0.69±0.05
V	12 (3.7)	146	0.65-0.82	0.72±0.05
W	22 (6.8)	270	0.59-0.88	0.72±0.06
X	25 (7.7)	300	0.53-0.88	0.71±0.09
Y	20 (6.2)	225	0.59-0.82	0.66±0.06
Total	325(100)	3799	0.29-1.0	0.69 ±0.1

*MARI: Multiple Antibiotic Resistance Index, **SD: Standard Deviation. a: significant difference at P=0.0078, b: at P=0.0003, c: at P=0.0082 and d: at P=0.0065.

Table 4: Antimicrobial resistant patterns of MARI of 325 ExPEC isolates recovered in North of Palestine.

Antibiotic resistant pattern	MARI	Number of resistant isolates (%)
5	0.29	1 (0.31)
6	0.35	2 (0.62)
7	0.41	3 (0.92)
8	0.47	6 (1.85)
9	0.53	13 (4.0)
10	0.59	36 (11.08)
11	0.65	85 (26.15)
12	0.71	85 (26.15)
13	0.76	52 (16.00)
14	0.82	27 (8.31)
15	0.88	14 (4.31)
17	1	1 (0.31)
Total		325 (100)

DISCUSSION

In countries with limited resources such as Palestine, antimicrobial agents are widely used in poultry farms for the prevention of infections and growth promotion. Continuous exposure to antimicrobial agents results in bacterial selection and the acquisition of resistance genes from different microbes, leading to the evolution of new strains with various resistance characteristics (Jalil *et al*., 2023). The resistance patterns of bacterial isolates vary according to the farms and are likely to be associated with the frequent use of specific antimicrobial agents in different farms. The classes of antibiotics including aminopenicillin (amoxicillin), cephalosporins (ceftiofur, ceftriaxone), fluoroquinolones (enrofloxacin, levofloxacin, norfloxacin), aminoglycosides (gentamicin), and amphenicols (florfenicol) demonstrated the highest resistance in the current study, indicating that these antibiotics were overused or misused in the treatment of microbial infections in local chicken farms and rendering these antibiotics ineffective in veterinary medicine in Palestine. Antimicrobial therapy has been identified as a crucial factor in reducing economic losses related to colibacillosis. This study revealed that ExPEC isolates exhibited high resistance to antibiotics commonly used in human therapy, including cephalosporins, fluoroquinolones, aminoglycosides, sulfonamides, aminopenicillins, and macrolides. Due to the potential transmission of resistance genes to human microorganisms, resistance in poultry bacteria may pose a threat to public health.

The study showed that the antibiotic resistance of the investigated ExPEC isolates was very high, with all isolates (100%) demonstrating multidrug resistance (resistant to more than 2 antibiotic classes). The prevalence of MDR was detected in several geographical areas in previously published studies, as it was 100% in China (Li *et al*., 2015; Afayibo *et al*., 2022; Dong *et al*., 2025), 94 and 91.6% in Napal (Subedi *et al*., 2018; Bhattarai *et al*., 2024), 100% in Egypt (Amer *et al*., 2018; El seedy *et al*., 2019), 41% in Italy (Sgariglia *et al*., 2019), 100% in Pakistan (Azam *et al*., 2019 ; Jalil *et al*., 2023), 60.7 and 100% in Thailand (Thomrongsuwannakij *et al*., 2020; Tongkamsai and Nakbubpa, 2024),), 10.7% in Australia (Thomrongsuwannakij *et al*., 2020), 95.9 and 52.5% in Algeria (Benklaouz *et al*., 2020; Aberkane *et al*., 2023), 96% in Tunisia (Dhaouadi *et al*., 2020), 90% in South Korea (Yoon *et al*., 2020), 60% in Brazil (Pilati *et al*., 2024) and 41.9% in Germany (Müller *et al*., 2024. The 325 ExPEC isolates revealed 188 antibiotic-resistant patterns. This result showed a wide variety in the distribution of resistance patterns among ExPEC isolates, explaining the observed high resistance profiles with the potential spread of antibacterial resistance determinants among ExPEC. The coexistence of virulence factors and multidrug-resistant antibiotics was common in the APEC isolates recovered in Palestine (Adwan *et al*., 2024; Abuseir *et al*., 2025). Generally, the results showed that this resistance of ExPEC is a cause for concern, as there are limited solutions for antibiotic treatment in the chicken industry.

The MARI variable was calculated for each farm, revealing significant differences in MARI values between farms A vs. I, I vs. K, J vs. K, and K vs. W. These variations may be attributed to restrictions imposed during medication usage. The highest MARI value found among isolates was 1.0 (1 isolate), while the lowest was 0.29 (1 isolate). The lowest average MARI value across farms was 0.56 ± 0.12, while the highest one was 0.77 ± 0.08 with an overall average MARI value for all isolates 0.69 ± 0.1.

In total, the highest number of isolates exhibited resistance to 11 and 12 antimicrobials with a MARI of 0.65 (26.15%, n = 85) and 0.71 (26.15%, n = 85), respectively, followed by 13 antimicrobials with a MARI of 0.76 (16.00%, n = 52). These findings indicate that the isolates are high-risk, likely originating from an environment with heavy antibiotic use. The high MARI in these farms suggests that the misuse and abuse of antimicrobials may be a fundamental feature to the prevalence of antibacterial-resistant ExPEC isolates in poultry farms in North Palestine (Saeed *et al*., 2023).

Tetracyclines are commonly used in veterinary medicine. The prevalence rate of ExPEC resistance to the tetracycline class was 43.1% for doxycycline in our study. Resistance to doxycycline has been detected in several countries in previously published studies. The prevalence of resistance to doxycycline among ExPEC isolates was 69.8, 85, 36.4 and 72.4% in Egypt (Younis *et al*., 2017; Amer *et al*., 2018; El seedy *et al*., 2019; Radwan *et al*., 2019), 92.9% in Jordan (Ibrahim *et al*., 2019), 82% in Tunisia (Dhaouadi *et al*., 2020), 87.5 and 76.4% in Nepal (Sankhi *et al*., 2020; Bhattarai *et al*., 2024), 93.9% in China (Afayibo *et al*., 2022), 91.7% in Algeria (Aberkane *et al*., 2023), 45.6 and 78.6% in Pakistan (Jalil *et al*., 2023; Saeed *et al*., 2023), 49.2% in Palestine (Adwan *et al*., 2024) and 45.5% in Thailand (Tongkamsai and Nakbubpa, 2024).

Fluoroquinolones serve as significant antibiotics used to treat pathogenic bacteria causing infections in both humans and animals. *E. coli* strains resistant to fluoroquinolones have been identified in both humans and animals worldwide (Misumi *et al*., 2023). Fluoroquinolones usually inhibit DNA synthesis process in bacteria. These antibiotics possess several desirable properties that have led to their widespread use in preventing and managing bacterial infections: good oral bioavailability, potent activity against a wide variety of bacterial species, a broad spectrum of indications for use, and favorable pharmacokinetic features. The ExPEC isolates demonstrated a high resistance rate to fluoroquinolones-class antibiotics. The resistance rates to enrofloxacin, levofloxacin, norfloxacin, and ciprofloxacin were 99.1%, 94.2%, 90.5%, and 84.9%, respectively. Resistance to these antibiotics has been reported in various regions of the world in previously published studies, with results that were either consistent or contradictory to those obtained in this study. The prevalence of isolates resistant to ciprofloxacin among ExPEC isolates was 94.3 and 67.1% in China (Li *et al*., 2015; Dong *et al*., 2025), 41.4, 81.8 and 95.9% in Egypt (Awad *et al*., 2016; El seedy *et al*., 2019; Radwan *et al*., 2019), 63.9% in Jordan (Ibrahim *et al*., 2019), 18% in Italy (Sgariglia *et al*., 2019), 72.0, 75.5 and 11.6% in Pakistan (Azam *et al*., 2019; Jalil *et al*., 2023; Saeed *et al*., 2023), 46.8% in South Korea (Kim *et al*., 2020), 72.4 and 87.5% in Algeria (Benklaouz *et al*., 2020; Aberkane *et al*., 2023), 82.5 and 67.5% in Nepal (Sankhi *et al*., 2020; Bhattarai *et al*., 2024), 100% in Brazil (Kimura *et al*., 2021), 71.7% in India (Limbachiya *et al*., 2022), 15.8% in Japan (Misumi *et al*., 2023), 86.2% in Palestine (Adwan *et al*., 2024) and 5.6% in Germany (Müller *et al*., 2024). The prevalence of ExPEC isolates resistance to norfloxacin was 89.7 and 57.7% in China (Li *et al*., 2015; Dong *et al*., 2025), 81.8% in Egypt (El seedy *et al*., 2019), 72.4% in Algeria (Benklaouz *et al*., 2020), 100 and 4.3% in Brazil (Kimura *et al*., 2021; Pilati *et al*., 2024), 59.2 and 43.3% in Pakistan (Jalil *et al*., 2023; Saeed *et al*., 2023) and 89.2% in Palestine (Adwan *et al*., 2024). The occurrence rate of enrofloxacin resistance among ExPEC isolates was 75.0 and 81.8% in Egypt (Amer *et al*., 2018; El seedy *et al*., 2019), 18% in Italy (Sgariglia *et al*., 2019), 80.7% in Algeria (Benklaouz *et al*., 2020), 96.1% in China (Afayibo *et al*., 2022), 100 and 39.4% in Brazil (Kimura *et al*., 2021; Pilati *et al*., 2024), 71.3% in Pakistan (Saeed *et al*., 2023), 98.5% in Palestine (Adwan *et al*., 2024) and 0.0% in Nepal (Bhattarai *et al*., 2024). The prevalence of resistance to levofloxacin among ExPEC isolates was 94.3% in China (Li *et al*., 2015), 42.4% in Egypt (Younis *et al*., 2017), 72.5 and 67.1% in Nepal (Sankhi *et al*., 2020; Bhattarai *et al*., 2024), 81.7% in India (Limbachiya *et al*., 2022) and 66.7% in Pakistan (Jalil *et al*., 2023). Resistance to this class of antimicrobials has developed due to its extensive use in the chicken industry, increasing the likelihood of treatment failure (Li *et al*., 2015; Maris *et al*., 2021). Additionally, fluoroquinolone resistance has become more common in ExPEC isolates in recent years, as fluoroquinolone-resistant ExPEC isolates often exhibit MDR phenotypes (Temmerman *et al*., 2020; Yoon *et al*., 2020; Jalil *et al*., 2023). This investigation revealed fluoroquinolone-resistant ExPEC isolates resistant to penicillin and tetracycline, consistent with previously published results (Temmerman *et al*., 2020; Yoon *et al*., 2020; Jalil *et al*., 2023).

Aminoglycoside antibiotics are a class used to treat severe infections in both humans and animals. They are generally combined with other antibiotics, often a β-lactam, due to their fast bactericidal effects. These antibiotics block bacterial protein synthesis by binding to the 30S subunit of the ribosome. The findings of this research showed that the resistance rate among ExPEC isolates to the aminoglycoside class was 92.9% for gentamicin and 45.3% for neomycin. The occurrence of resistance rates among ExPEC isolates to gentamicin has been reported in several parts of the world. These results were either in consensus with or in disagreement with the results obtained in this study. Specifically, the resistance rates were reported as 93.1, 62.6, and 31.5% in China (Li *et al*., 2015; Afayibo *et al*., 2022; Dong *et al*., 2025), 48.3, 55, 45.5, 89.9% in Egypt (Awad *et al*., 2016; Amer *et al*., 2018; El Seedy *et al*., 2019; Radwan *et al*., 2019), 6% in Italy (Sgariglia *et al*., 2019), 57.2% in Jordan (Ibrahim *et al*., 2019), 1.2% in Australia (Thomrongsuwannakij *et al*., 2020), 32.5 and 31.0% in Nepal (Sankhi *et al*., 2020; Bhattarai *et al*., 2024), 13.5 and 32.5% in Algeria (Benklaouz *et al*., 2020; Aberkane *et al*., 2023), 95.2% in Thailand (Thomrongsuwannakij *et al*., 2020), 61.9 and 30.2% in Brazil (Kimura *et al*., 2021; Pilati *et al*., 2024), 23.3% in India (Limbachiya *et al*., 2022), 57.3% in Pakistan (Saeed *et al*., 2023), 80% in Palestine (Adwan *et al*., 2024) and 5.6% in Germany (Müller *et al*., 2024). The rate of resistance among ExPEC isolates to neomycin that was documented in several regions was 52.9, and 43.8% in China (Li *et al*., 2015; Dong *et al*., 2025), 89.0% in Egypt (Younis *et al*., 2017), 80.7% in Algeria (Benklaouz *et al*., 2020), 12.2% in Pakistan (Saeed *et al*., 2023), 61.5% in Palestine (Adwan *et al.,* 2024) and 47.0% in Nepal (Bhattarai *et al*., 2024).

Synthetic antimicrobials called trimethoprim and sulfonamides block distinct stages of the folic acid synthesis pathway. Each of these compounds has bacteriostatic characteristics, but when sulfonamide and trimethoprim are combined, they produce synergistic bactericidal effects on vulnerable microorganisms. The rate of resistance of ExPEC isolates to the sulfonamide class was 80% for trimethoprim/sulfamethoxazole. The resistance rate among ExPEC isolates to sulfamethoxazole reported in several countries was 58.6, 90.4, and 85.7% in Egypt (Awad *et al*., 2016; Younis *et al*., 2017; Radwan *et al*., 2019), 41% in Italy (Sgariglia *et al*., 2019), 95.5% in Jordan (Ibrahim *et al*., 2019), 57.6% in South Korea (Yoon *et al*., 2020), 17.9% in Australia (Thomrongsuwannakij *et al*., 2020), 52.0% in Tunisia (Dhaouadi *et al*., 2020), 73.9 and 62.5% in Algeria (Benklaouz *et al*., 2020; Aberkane *et al*., 2023), 51.2 and 100% in Thailand (Thomrongsuwannakij *et al*., 2020; Tongkamsai and Nakbubpa, 2024), 76.2 and 42.9% in Brazil (Kimura *et al*., 2021; Pilati *et al*., 2024), 84.3% in China (Afayibo *et al*., 2022), 80.9 and 68.9% in Pakistan (Jalil *et al*., 2023; Saeed *et al*., 2023), 78.5% in Palestine (Adwan *et al*., 2024) and 56.3% in Nepal (Bhattarai *et al*., 2024). The results of our investigation were either comparable to or different from those found in other states.

The high resistance rate of ExPEC to aminopenicillin class was 100% for amoxicillin; to the cephalosporins class, it was 99.4% for ceftiofur, 95.4% for ceftriaxone, and 67.4% for cephalexin. The prevalence of ceftriaxon resistance among ExPEC isolates in several regions was 88.5 and 57.7% in China (Li *et al*., 2015; Dong *et al*., 2025), 4.8% in Jordan (Ibrahim *et al*., 2019), 45.5 and 89.9% in Egypt (El Seedy *et al*., 2019; Radwan *et al*., 2019), 15% in India (Limbachiya *et al*., 2022), 17.7% in Pakistan (Jalil *et al*., 2023), 0.0% in Algeria (Aberkane *et al*., 2023), 89.2% in Palestine (Adwan *et al*., 2024), 25.7% in Nepal (Bhattarai *et al*., 2024) and 44.4% in Brazil (Pilati *et al*., 2024). The incidence of ceftiofur resistance among ExPEC isolates was 3.4% in Algeria (Benklaouz *et al*., 2020), 20% in Tunisia (Dhaouadi *et al*., 2020), 92.3% in Palestine (Adwan *et al*., 2024), and 44.4% in Brazil (Pilati *et al*., 2024). The prevalence of cephalexin resistance among ExPEC isolates in a recent study in Palestine was 72.3% (Adwan *et al*., 2024). The occurrence of amoxicillin resistance among ExPEC isolates was 94.5% and 100% in Egypt (Younis *et al*., 2017; El Seedy *et al*., 2019), 93.3% in Jordan (Ibrahim *et al*., 2019), 82.7% in Algeria (Benklaouz *et al*., 2020), 78% in Tunisia (Dhaouadi *et al*., 2020), 29.8% in Australia (Thomrongsuwannakij *et al*., 2020), 70.2 and 90.9% in Thailand (Thomrongsuwannakij *et al*., 2020; Tongkamsai and Nakbubpa, 2024), 81.7 and 90.4% in China (Afayibo *et al*., 2022; Dong *et al*., 2025), 92.5% in Pakistan (Jalil *et al*., 2023) and 100% in Palestine (Adwan *et al*., 2024). Beta-lactamase-producing microbes break down antibiotics with β-lactam rings, including penicillin-related compounds and first- to fourth-generation cephalosporins, and aztreonam (Castanheira *et al*., 2021). Third-generation cephalosporins are essential in veterinary medicine, and third- and fourth-generation cephalosporins constitute significant antimicrobial agents in humans. Resistance to third- or fourth-generation cephalosporins is rapidly increasing in *E. coli*, attributed to the incidence of plasmid-encoded enzymes, such as extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC (pAmpC) β-lactamases. *E. coli* isolates harboring ESBLs and/or pAmpC β-lactamases are resistant to extended-spectrum cephalosporins, particularly third- or fourth-generation cephalosporins (Poirel *et al*., 2018; Kang *et al*., 2022). Due to their high cost, Palestinian poultry farms rarely utilize third- or fourth-generation cephalosporins. However, the third-generation cephalosporins Ceftriaxone and Ceftiofur showed significant resistance against these ExPEC isolates in Palestine. Therefore, the development of *blaCTX-M* genes in Palestinian broiler farms may have an effect on the horizontal gene transfer that spreads and transmits these genes among bacterial populations through plasmids, or the emergence of isolates that are resistant to third- and fourth-generation cephalosporins may occur independently of the administration of these antibiotics in farms. The usage of other antibiotics with β-lactam rings, like penicillin and its derivatives, can lead to β-lactamase gene development and selective resistance to third- and fourth-generation cephalosporins. According to penicillin and penicillin derivatives routinely used in chicken farms, the third- and fourth-generation of cephalosporins break down β-lactam rings due to the products of *blaCTX-M* genes (Kang *et al*., 2022; Yoon *et al*., 2020). In this study, the ExPEC isolates demonstrated total resistance to amoxicillin, which is one of the penicillin derivatives frequently used in Palestinian poultry farms. The β-lactam ring in penicillin and its derivatives, including amoxicillin, is inactivated by the products of *blaTEM* genes. Plasmid-mediated quinolone resistance genes are the cause of the high resistance to ciprofloxacin, norfloxacin, and enrofloxacin. The existence of plasmid-mediated quinolone resistance genes might be closely associated with β-lactamase genes, potentially because they are carried together on a plasmid in Enterobacteriaceae (Yoon *et al*., 2020). As a result, it is essential to monitor ESBL genes to observe the spread of these genes across various reservoirs in the poultry sector.

The current research results showed that the resistance rate of ExPEC to the amphenicols class was 90.2% for florfenicol. The prevalence of resistance among ExPEC isolates to florfenicol was 93.7% in Jordan (Ibrahim *et al*., 2019), 56% in Tunisia (Dhaouadi *et al*., 2020), 3.8% in Australia (Thomrongsuwannakij *et al*., 2020), 69.6 and 89.0% in China (Afayibo *et al*., 2022; Dong *et al*., 2025), 84.6% in Palestine (Adwan *et al*., 2024) and 0.9% in Germany (Müller *et al*., 2024). The results of our research were either comparable to or different from those reported in other nations. The high rate of resistance of ExPEC isolates toward the tested antibiotics may be due to their frequent use in the clinical management of avian colibacillosis. An analogue of chloramphenicol used in veterinary medicine, florfenicol has a very broad range of activity and targets the synthesis of bacterial proteins. Due to its potent therapeutic success against bacterial infections in animals, particularly poultry, it is usually used in veterinary clinics as a feed additive and therapy.

Detection colistin sensitivity promptly and accurately is therefore essential. Broth microdilution is presently considered the gold standard method, while agar diffusion methods are not recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Bentaher *et al*., 2025). However, colistin is still evaluated using the agar diffusion methods (El Seedy *et al*., 2019; Dhaouadi *et al*., 2020; Benklaouz *et al*., 2020; Tofani *et al*., 2022; Limbachiya *et al*., 2022; Aberkane *et al*., 2023; Thabet*et al*., 2023; Saeed *et al*., 2023; Adwan *et al*., 2024). Results also showed that the polymyxins class (polymyxins E (colistin) and polymyxin B) and aminocyclitol class (spectinomycin) were the most effective drugs against Palestinian ExPEC isolates, with a resistance rate of 10%, 22.1%, and 26.8%, respectively. Colistin is legally restricted for use in poultry therapy due to serious concerns that resistance to colistin may be passed from poultry to people, as it is used to treat human infections caused by Gram-negative bacteria. However, this medicine is being utilized illegally in chicken farms. The prevalence resistance rate of colistin among ExPEC isolates was 36.4% in Egypt (El seedy *et al*., 2019), 0.0 and 14.4% in Algeria (Benklaouz *et al*., 2020; Aberkane *et al*., 2023), 24% in Tunisia (Dhaouadi *et al*., 2020), 0.0% in Italy (Tofani *et al*., 2022), 0.0% in India (Limbachiya *et al*., 2022), 4.0 and 70%

in Pakistan (Jalil *et al*., 2023; Saeed *et al*., 2023), 37.5 and 27.7% in Palestine (Thabet *et al*., 2023; Adwan *et al*., 2024), 4.7% in Nepal (Bhattarai *et al*., 2024) and 2.8% in Germany (Müller *et al*., 2024). The incidence of resistance among ExPEC isolates toward polymyxin B was 0.4 and 5.5% in China (Afayibo *et al*., 2022; Dong *et al*., 2025), 55.4% in Palestine (Adwan *et al*., 2024) and 81.8% in Thailand (Tongkamsai and Nakbubpa, 2024). The occurrence of resistance among ExPEC isolates to spectinomycin was 23 and 34.3% in China (Li *et al*., 2015; Dong *et al*., 2025), 15% in India (Limbachiya *et al*., 2022), and 92.2% in Palestine (Thabet *et al*., 2023). These results were either in consensus with or in disagreement with the results obtained in our research. Polymyxins exert their antibacterial effects primarily by targeting the lipopolysaccharide present in Gram-negative bacteria's outer membrane, through the membrane lysis death pathway. However, this is not adequate to clarify the observed lethality applied by them. Several lesser mechanisms undoubtedly play a role in the general inhibitory action of the polymyxins, such as vesicle-vesicle contact pathway, ribosome binding, effect on cell division, Gram-positive secretion system inhibition of bacterial respiration, and hydroxyl radical death pathway. One of the primary mechanisms through which bacteria develop resistance involves alterations in the lipopolysaccharide structure, it was found that the complete loss of lipopolysaccharide could lead to high-level polymyxin E resistance, overexpression of efflux pumps, intrinsic resistance to polymyxins, and expression of outer membrane proteins increases the expression of capsule polysaccharide, thereby decreasing susceptibility to polymyxin B in certain bacteria (Yu *et al*., 2015; Mohapatra *et al*., 2021). Chromosome gene mutations or the acquisition of resistance gene mutations might be the source of colistin resistance (Poirel *et al*., 2018). Colistin is used frequently in the treatment of animals. Because of its extensive incidence across the world, it might render polymyxins unsuccessful if immediate corrective actions are not taken to prevent the utilization of polymyxins in animal production. The resistant rate of spectinomycin was 26.8%, which may be due to its rare use in clinical veterinary medicine because of its high price. Spectinomycin is considered a wide-spectrum antimicrobial that is a member of the aminocyclitol class. Its function is carried out by inhibiting bacterial protein synthesis.

The resistance rate of ExPEC isolates to the phosphonic antibiotic class was 40.3% for fosfomycin. The prevalence rate of resistance among ExPEC isolates to fosfomycin was 30% in Jordan (Ibrahim *et al*., 2019) and 35.4% in Palestine (Adwan *et al*., 2024). The peptidoglycan synthesis-related enzyme MurA gets inhibited by fosfomycin. In veterinary medicine, fosfomycin is primarily used to treat infections in broiler chickens that occurred on by a variety of bacterial species belonging to Gram-positive and Gram-negative pathogens, including *E. coli*.

In general, it has been widely recognized that bacteria acquired different resistance mechanisms to antibiotics such modification in the drug-binding site as mutations in drug specific target genes, multidrug efflux systems or efflux transporters to reduce the drug concentration, reduced permeability of the outer membrane, alteration or deactivation of the drug by specific modifying enzymes expressed on chromosomes or plasmids, resistance genes associated with antibiotic, acquisition of genes encoding enzymes that are insensitive to antibiotic, mutations that change randomly bacterial chromosome, which can lead to cross-resistance and acquisition of antibiotic resistance genes. Through horizontal gene transfer, most of the genes coexisted with bacterial mobile genetic elements, such as integrons, transposons, or plasmids, which supported the quick dissemination of antibiotic resistance genes across several bacterial species (Poirel *et al*., 2018; Kim *et al*., 2020; Hasan and Al-Harmoosh, 2020; Li *et al*., 2020).

Conclusions: Antibiotic resistance was found to be common among ExPEC isolates collected from poultry farms in Palestine, including resistance to β-lactams, quinolones, aminoglycosides, amphenicols, and macrolides. The improper use of antibiotics for growth promotion and treatment of infections in animals is a widespread practice in Palestine and other nations. This highlights how the indiscriminate and abusive use of various antibacterial agents for prevention or treatment leads to the emergence of drug-resistant bacterial strains.

Alternative methods, such as immunization, have become more popular due to the need to manage ExPEC at the flock level without relying on antibiotics, which should be reserved to mitigate the impacts of antibiotic overuse and misuse. Research into antibiotic resistance in ExPEC is crucial to understand the efficacy of antibiotic treatment and to improve the effective control strategies. A comprehensive strategy is essential to successfully prevent and manage avian colibacillosis in Palestine.

Acknowledgements: The authors would like to acknowledge An-Najah National University (ANNU) for facilitating the implementation of this research.

Authors contributions: SA, GO, MA and GA: Conceptualization, methodology, investigation, data curation, drafted and revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

Financial Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Aberkane C, Messaï A, Messaï CR, *et al*., 2023. Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. Veterinary World 16:449-54.

Abuseir S, Omar G, Albzour M, *et al*., 2025. Typing Beta-lactamase-producing avian pathogenic *Escherichia coli* isolates recovered from broiler farms in Northern Palestine. Pakistan Veterinary Journal 45(3): 1369-76.

Adwan G, Abuseir S, Omar G, *et al*., 2024. Molecular characterization of avian pathogenic *Escherichia coli* isolates from broiler farms in Northern Palestinian territories. Veterinary World 17:2865-79.

Adwan G, Adwan K, Jarrar N, *et al*., 2013. Prevalence of *seg, seh* and *sei* genes among clinical and nasal swab of *Staphylococcus aureus* isolates. British Microbiology Research Journal 3:139-49.

Afayibo DJA, Zhu H, Zhang B, *et al*., 2022. Isolation, molecular characterization, and antibiotic resistance of avian pathogenic *Escherichia coli* in Eastern China. Veterinary Sciences 9:319.

Amer MM, Mekky HM, Amer AM, *et al*., 2018. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Veterinary World 11:1082-8.

Awad A, Arafat N and Elhadidy M, 2016. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. Annals of Clinical Microbiology and Antimicrobials 15:59.

Azam M, Mohsin M, Sajjad-Ur-Rahman, *et al*., 2019. Virulence-associated genes and antimicrobial resistance among avian pathogenic *Escherichia coli* from colibacillosis affected broilers in Pakistan. Tropical Animal Health and Production 51:1259-65.

Benklaouz MB, Aggad H and Benameur Q, 2020. Resistance to multiple first-line antibiotics among *Escherichia coli* from poultry in Western Algeria. Veterinary World 13:290-5.

Bentaher I, Sessouma A, Lahlou YB, *et al*., 2025. Methods for assessing *in vitro* susceptibility to colistin: to what extent could the disk diffusion method be considered useful? Saudi Journal of Medical and Pharmaceutical Sciences 11: 692-6.

Bhattarai RK, Basnet HB, Dhakal IP, *et al*., 2024. Antimicrobial resistance of avian pathogenic *Escherichia coli* isolated from broiler, layer, and breeder chickens. Veterinary World 17: 480-99.

Castanheira M, Simner PJ and Bradford PA, 2021. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-Antimicrob Resistance 3:dlab092.

Clinical and Laboratory Standards Institute (CLSI), 2023. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100 (ISBN 978-1-68440-170-3 [Print]; ISBN 978-1-68440-171-0 [Electronic]). Clinical and Laboratory Standards Institute, USA.

Dhaouadi S, Soufi L, Hamza A, *et al*., 2020. Co-occurrence of mcr-1 mediated colistin resistance and β-lactamase-encoding genes in multidrug-resistant *Escherichia coli* from broiler chickens with colibacillosis in Tunisia. Journal of Global Antimicrobial Resistance 22:538-45.

Dong H, Zhu S, Zheng D, *et al*., 2025. Antibiotic resistance and molecular typing of duck-derived *Escherichia coli* isolates. Comparative Immunology, Microbiology and Infectious Diseases 118:102321.

El Seedy FR, Abed AH, Wafaa MMH, *et al*., 2019. Antimicrobial resistance and molecular characterization of pathogenic *E. coli* isolated from chickens. Journal of Veterinary Medicine Research 26:280-92.

Hasan TH and Al-Harmoosh RA, 2020. Mechanisms of antibiotics resistance in bacteria. Systematic Reviews in Pharmacy 11:817-23.

Ibrahim RA, Cryer TL, Lafi SQ, *et al*., 2019. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Veterinary Research 15:159.

Jalil A, Masood S, Ain Q, *et al*., 2023. High resistance of fluoroquinolone and macrolide reported in avian pathogenic *Escherichia coli* isolates from the humid subtropical regions of Pakistan. Journal of Global Antimicrobial Resistance 33:5-17.

Kang HJ, Lim SK and Lee YJ, 2022. Genetic characterization of third- or fourth-generation cephalosporin-resistant avian pathogenic *Escherichia coli* isolated from broilers. Frontiers in Veterinary Science 9: 1055320.

Karpov DS, Kazakova EM, Kovalev MA, *et al*., 2024. Determinants of antibiotic resistance and virulence factors in the genome of *Escherichia coli* APEC 36 strain isolated from a broiler chicken with generalized colibacillosis. Antibiotics (Basel) 13:945.

Kim YB, Yoon MY, Ha JS, *et al*., 2020. Molecular characterization of avian pathogenic *Escherichia* coli from broiler chickens with colibacillosis. Poultry Science 99:1088-95.

Kimura AH, Koga VL, de Souza Gazal LE, *et al*., 2021. Characterization of multidrug-resistant avian pathogenic *Escherichia coli*: an outbreak in canaries. Brazilian Journal of Microbiology 52:1005-12.

Krumperman PH, 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology 46:165-70.

Li P, Zhu T, Zhou D, *et al*., 2020. Analysis of resistance to florfenicol and the related mechanism of dissemination in different animal-derived bacteria. Frontiers in Cellular and Infection Microbiology 10:369.

Li Y, Chen L, Wu X, *et al*., 2015. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Poultry Science 94:601-11.

Limbachiya BB, Mathakiya RA, Patel KJ, *et al*., 2022. Determination of antibiotic susceptibility of avian pathogenic *Escherichia coli* by phenotypic and genotypic methods. Indian Journal of Veterinary Sciences and Biotechnology 18:26-31.

Maris AS, Mody P, Brewer DJ, et al., 2021. The Fluoroquinolones: An update for the clinical microbiologist. Clinical Microbiology Newsletter 43:97-107.

Misumi W, Magome A, Okuhama E, *et al*., 2023. CTX-M-55-type ESBL-producing fluoroquinolone-resistant *Escherichia coli* sequence type 23 repeatedly caused avian colibacillosis in Kagoshima Prefecture, Japan. Journal of Global Antimicrobial Resistance 35:325-31.

Mohapatra SS, Dwibedy SK and Padhy I, 2021. Polymyxins, the last-resort antibiotics: Mode of action, resistance emergence, and potential solutions. Journal of Biosciences 46:85.

Müller A, Schulze Bernd K, Seinige D, *et al*., 2024. Molecular characterization of *Escherichia coli* isolates recovered from broilers with cellulitis. Poultry Science 103:103704.

Pilati GVT, Salles GBC, Savi BP, *et al*., 2024. Isolation and characterization of *Escherichia coli* from Brazilian broilers. Microorganisms. 12:1463.

Poirel L, Madec JY, Lupo A, *et al*., 2018. Antimicrobial resistance in *Escherichia coli*. Microbiology Spectrum 6:10.1128/microbiolspec.arba-0026-2017.

Radwan IA, Tamam SM, El Sayed AA, *et al*., 2019. Antimicrobial patterns of avian pathogenic *Escherichia coli* isolated from broiler chickens. Journal of Veterinary Medicine and Research 26:134-42.

Saeed MA, Saqlain M, Waheed U, *et al*., 2023. Cross-sectional study for detection and risk factor analysis of ESBL-Producing avian pathogenic *Escherichia coli* associated with backyard chickens in Pakistan. Antibiotics (Basel) 12:934.

Saidenberg ABS, Edslev SM, Hallstrøm S, *et al*., 2024. *Escherichia coli* ST117: exploring the zoonotic hypothesis. Microbiology Spectrum 12:e0046624.

Sankhi S, Bhattarai RK, Basnet HB, *et al*., 2020. Identification of antibiotic resistance pattern, and detection of virulence genes *iss,* and *ompT* in avian pathogenic *Escherichia coli* from broiler chickens in Chitwan, Nepal. Journal of Pharmaceutical Research International 32(23):1-12.

Sgariglia E, Aconiti Mandolini N, Napoleoni M, *et al*., 2019. Antibiotic resistance pattern and virulence genes in avian pathogenic *Escherichia coli* (APEC) from different breeding systems. Veterinaria Italiana 55:26-33.

Subedi M, Luitel H, Devkota B, *et al*., 2018. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. BMC Veterinary Research 14:113.

Temmerman R, Garmyn A, Antonissen G, *et al*., 2020. Evaluation of fluoroquinolone resistance in clinical avian pathogenic *Escherichia coli* isolates from Flanders (Belgium). Antibiotics (Basel) 9:800.

Thabet AM, Alzuheir IM, Laham NAA, *et al*., 2023. First report of mobile colistin resistance gene *mcr*-1 in avian pathogenic *Escherichia coli* isolated from turkeys in the Gaza Strip, Palestine. Veterinary World 16:1260-5.

Thomrongsuwannakij T, Blackall PJ, Djordjevic SP, *et al*., 2020. A comparison of virulence genes, antimicrobial resistance profiles and genetic diversity of avian pathogenic *Escherichia coli* (APEC) isolates from broilers and broiler breeders in Thailand and Australia. Avian Pathology 49:457-66.

Tofani S, Albini E, Blasi F, *et al*., 2022. Assessing the load, virulence and antibiotic-resistant traits of ESBL/Ampc *E. coli* from broilers raised on conventional, antibiotic-free, and organic farms. Antibiotics (Basel) 11:1484.

Tongkamsai S and Nakbubpa K, 2024. Extended-spectrum beta-lactamase (ESBL) production and virulence genes profile of avian pathogenic *Escherichia coli* (APEC) isolated from broiler chickens in eastern Thailand. Veterinary Integrative Sciences 22:207-18.

Watts A and Wigley P, 2024. Avian pathogenic *Escherichia coli*: An overview of infection biology, antimicrobial resistance and vaccination. Antibiotics (Basel) 13:809.

Yoon MY, Kim YB, Ha JS, *et al*., 2020. Molecular characteristics of fluoroquinolone-resistant avian pathogenic *Escherichia coli* isolated from broiler chickens. Poultry Sciences 99:3628-36.

Yoon S and Lee YJ, 2022. Molecular characteristics of ESBL-producing *Escherichia coli* isolated from chickens with colibacillosis. Journal of Veterinary Science 23:e37.

Younis G, Awad A and Mohamed N, 2017. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *Escherichia coli* isolated from broiler chickens. Veterinary World 10:1167-72.

Yu Z, Qin W, Lin J, *et al*., 2015. Antibacterial mechanisms of polymyxin and bacterial resistance. BioMed Research International 2015:679109.