Antibiotic Resistance Pattern of Avian Pathogenic *Escherichia Coli* O78 in Turkey Poults

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Abstract

Avian pathogenic *Escherichia coli* (APEC) is responsible for a range of systemic and localized infections, presenting with varied clinical manifestations including coli granuloma, polyserositis, air vasculitis, and septicemia. Colibacillosis leads to substantial financial losses in the poultry sector, as it contributes to poultry deaths, poor feed efficiency, and contamination of carcasses, all of which necessitate disease management efforts. In this research, 30 *E. coli* strains were isolated from 50 diseased turkey poults. All bacterial isolates underwent antimicrobial susceptibility testing, and serotyping was conducted on thirty multidrug-resistant (MDR) strains. A significant level of resistance was most commonly detected against doxycycline, chloramphenicol, and amoxicillin-clavulanic acid. Multidrug resistance was found at a rate of 83.33% (25/30). Of the 30 strains successfully serotyped, the most commonly identified serogroups were *O78* and *O1*, each accounting for 20% (6 out of 30) of the total, while *O18, O113,* and *O119* rate was 10%. In the same time *O103, O111, O25* and *O26* rate was 6.6% detected as rare serogroups. The *O78* serotype was identified as both a predominant type within the selected *E. coli* isolates and exhibited elevated resistance to antibiotics. Five *O78* were subjected for detection of four resistance genes *blaTEM*, *floR*, *mph* A and *tet*A (A). All five have *tet*A (A) and *floR* while four isolates have *blaTEM* and *mph* A.

Keywords: Antibiotic resistance, Colibacillosis, *E. coli O78*

1. Introduction

The emergence of antibiotic resistance in avian pathogenic *Escherichia coli* (APEC), particularly the *O78* serotype, has significant implications for poultry health and public safety. As turkey poults are increasingly subjected to antibiotic treatments, the subsequent evolutionary pressures have led to the selection of resistant bacterial strains, which complicates treatment protocols and heightens the risk of transmission to humans. Understanding the patterns of antibiotic resistance in *E. coli O78* is critical, as it not only influences the health and productivity of the poultry industry but also represents a possible threat to food safety and societal health through the food chain. Moreover, the global rise in antibiotic resistance has raised concerns among veterinarians and public health officials, necessitating a comprehensive analysis of resistance profiles and underlying mechanisms. This research aims to elucidate the extent of antibiotic tolerance in APEC *O78* isolates from turkey poults, thereby contributing to mitigating the challenges posed by this growing public health issue.

Avian pathogenic *Escherichia coli* is a critical concern in the poultry sector, contributing to substantial economic losses and animal welfare issues. One serotype of particular interest is *E. coli O78*, which has been related to disease outbreaks in turkey poults. Poultry, including turkeys, have been implicated as potential reservoirs for antibiotic-resistant *Enterococcus* spp., which is potentially harmful to human health ***(Osman et al., 2019)***. Additionally, studies have found a high incidence of AmpC-producing *E. coli* and extended-spectrum beta-lactamase in healthy broiler chickens, highlighting the potential for transfer to individuals via food production chain. ***(Reich et al., 2013)***. Colibacillosis, a disease resulting from APEC, is a significant factor of mortality in caged layer hens, with outbreaks characterized by flock-level mortality rates reaching over 9% in some cases. ***(Vandekerchove et al., 2004)***. The ability of APEC to persist and spread within a production facility is a major challenge, with recurrent outbreaks common. ***(Vandekerchove et al., 2004)***.

The emergence of antibiotic resistance mechanisms in *Escherichia coli O78* poses a notable threat to poultry health and production, particularly among turkey poults. This strain of avian pathogenic *E. coli* (APEC) has developed various mechanisms to evade antibiotic efficacy, including the production of beta-lactamases, which degrade critical antibiotics, and alterations in cell permeability that limit drug access. Resistance plasmids can also be transferred between bacteria, facilitating the rapid spread of resistant traits across populations ***(Achtman et al.,***

2010). The appearance of antibiotic resistance in avian pathogenic *Escherichia coli* O78 poses significant challenges to both the health of turkey poults and the economic viability of poultry farms. As antibiotic resistance profiles evolve, the efficacy of commonly used therapeutic interventions diminishes, resulting in increased rates of illness and death among infected turkeys. This increased incidence of illness necessitates a greater use of alternative, often more expensive treatments, thereby straining farm economics. Additionally, co-infections with pathogens such as low pathogenic avian influenza can exacerbate the health complications associated with *E. coli* infections, further complicating management strategies and increasing production losses ***(Umar et al., 2017).*** This study targeted to describe the prevalence of *E. coli O78 in diseased turkey poults* and assess their antibiotic resistance and detection of resistance genes.

2. Material and methods

Bacterial Strains

During the period 2023 to 2024, altogether, 50 diseased turkey poults were randomly gathered from 5 turkey farms in Sharkia governorate, Egypt. From these samples, 30 bacterial isolates were obtained and identified, as *Escherichia coli* strains.

Sample collection and bacterial isolation and identification

The sample size was determined using a calculator provided by Survey Monkey (https://www.surveymonkey.com). Samples were gathered from visceral organ (spleen, liver, cecal tonsils, and gall bladder) of day old to30 days age old diseased poults. Routine bacteriological examination applied to collected samples for *Enterobactreace* isolation. Using enrichment broth media for cultivation of samples before plating. The samples were maintained in aerobic settings at 37°C for a 24-hour incubation period. Following incubation, the developed colonies underwent Gram staining along with catalase and oxidase assays to identify gram-negative bacilli exhibiting fermentative characteristics. The Gram-negative bacilli that tested negative for oxidase were re-inoculated onto MacConkey agar to obtain pure isolates and then recognized to the species level using standard biochemical approaches described by ***Finegold & Baron (1986)*** and GnA+B-ID System (Microgen Bioproducts, Ltd,AdmiraltWay, Camberley, SurreyGU15 3DT,U.K.), as directed by the manufacturer. Serological typing of *E. coli* isolates using group O-sera (DENKA SEIKEN, Tokyo, Japan) by employing slide agglutination tests as per ***Edwards & Ewing (1972)***.

Antimicrobial Susceptibility Testing

The bacterial isolates were evaluated in vitro to assess their

sensitivity to seven different antimicrobial compounds (Oxoid, Hampshire, UK). A Kirby–Bauer disc diffusion procedure was utilized, adhering to the guidelines and analytical standards set by the Clinical and Laboratory Standards Institute ***(CLSI, 2011).*** The subsequent antimicrobial agents were evaluated: amoxicillin–clavulanic acid, 30 µg; chloramphenicol, 30 µg; gentamicin ,10 µg. sulfamethoxazole–trimethoprim, 25 µg. colistin, 10 µg. and doxycycline, 30 µg. The isolates' susceptibility to antimicrobial agents was subdivided as susceptible, intermediate, or resistant by assessing the inhibition zone, based on interpretive criteria that followed the CLSI guidelines. The isolates that illustrated resistance to ≥ two various antimicrobial classes were divided as multidrug resistance.

DNA Extraction and Screening of Antimicrobial Resistance Genes

According to the instructions of the QIAamp DNA mini kit Catalogue no.51304, genomic DNA was extracted from bacterial isolates**.** The selected isolates were repeatedly tested for the occurrence of genes conferring resistance to erythromycin (*mphA)*, amoxicillin–clavulanic acid (bla_{TEM}), chloramphenicol (*flo*R), and doxycycline *(TetA(A)*. Polymerase chain reaction (PCR), target genes, and primer sequences products are depicted in Table 1.

Multiple PCR methods were employed to determine the target genes detected in the isolates, as outlined in Table 2. The amplified PCR fragments were electrophoresed on a 1.0% agarose gel (Sigma-Aldrich, St. Louis, MO, USA) pre-stained with ethidium bromide at a concentration of 0.5 µg/mL (Sigma-Aldrich, St. Louis, MO, USA). The amplified DNA samples were separated using electrophoresis at 100 volts for one hour on a compact horizontal gel system (Bio-Rad, Hercules, CA, USA). After electrophoresis, the gel was examined and imaged with UV light using a transilluminator.

Table (1): *Oligonucleotide primers sequences Source: Metabion (Germany).*

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
mphA	GTGAGGAGGAGCTTCGCGAG	403 bp	***Nguyen et al., 2009***
	TGCCGCAGGACTCGGAGGTC		
blaTEM	ATCAGCAATAAACCAGC	516 bp	***Colom et al., 2003***
	CCCCGAAGAACGTTTTC		
tetA(A)	GGTTCACTCGAACGACGTCA	570 bp	***Randall et al. 2004***
	CTGTCCGACAAGTTGCATGA		
floR	TTTGGWCCGCTMTCRGAC	494 bp	***Doublet et al., 2003***
	SGAGAARAAGACGAAGAAG		

Table (2): *Cycling conditions of the various primers through Cpcr*.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
mphA	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
bla_{TEM}	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec	72˚C 45 sec	35	72˚C 10 min.
TetA(A)	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec	72˚C 45 sec	35	72˚C 10 min.
floR	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec	72˚C 45 sec	35	72˚C 10 in.

3.Results

In this research 30 *E. coli* strains were isolated from 50 diseased turkey poults with prevalence rate 60% which were obtained from liver, gall bladder, cecal tonsils and lung of one-day old to 30 days old turkey poults collected from turkey poults farms in Sharkia government. Most *E. coli* isolated at 21-30 days old with 50% (15/30) followed by 11-20 days old turkey poults with 33.33%(10/30) most of them isolated from liver and gallbladder with 37.5% from each organ.

Biotyping results of *E. coli* isolate from turkey poults using GN24 system showing excellent *E. coli* identification (id%:99.97, Tindex:0.96).

Serotyping of some *E. coli* isolates

Slide agglutination testing was conducted on 30 *E. coli* isolates using targeted polyvalent and monovalent *E. coli* O antisera for serotyping purposes. Ten various serotypes were recognized among selected *E. coli* isolates, and the most widespread one was *E. coli O78* and *E. coli O1* with (6/30) 20% (**Table 3**).

Antibiogram of *E. coli*

Susceptibility of all 30 *E. coli* isolates was evaluated against various antimicrobial agents from multiple classes. Antibiogram of *E. coli* revealed that 80% (24/30) were resistant to Amoxicillin+ clavulanic acid, 83.3% (25/30) for chloramphenicol and 53.33% (16/30) to doxycycline. The isolates were highly sensitive for colistin with 100% (30/30), Gentamicin with 70% (21/30) and Trimethoprim sulfamethoxazole with 63.33% (19/30) as displayed in **table (4)**.

Antibiogram of *E. coli* O78

All five isolate were resistant to amoxicillin clavulanic acid, chloramphenicol and doxycycline in contrast all of them were sensitive to colistin. At the same time, four isolates were unresponsive to trimethoprim-sulfamethazole. Although all of them were multidrug resistance at least for two antimicrobial agents as displayed in table (5).

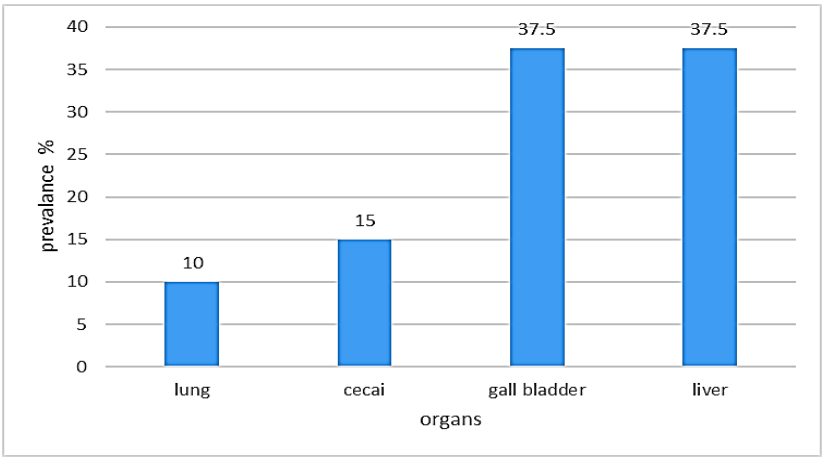

Figure (1) show the percent of isolates from different organ

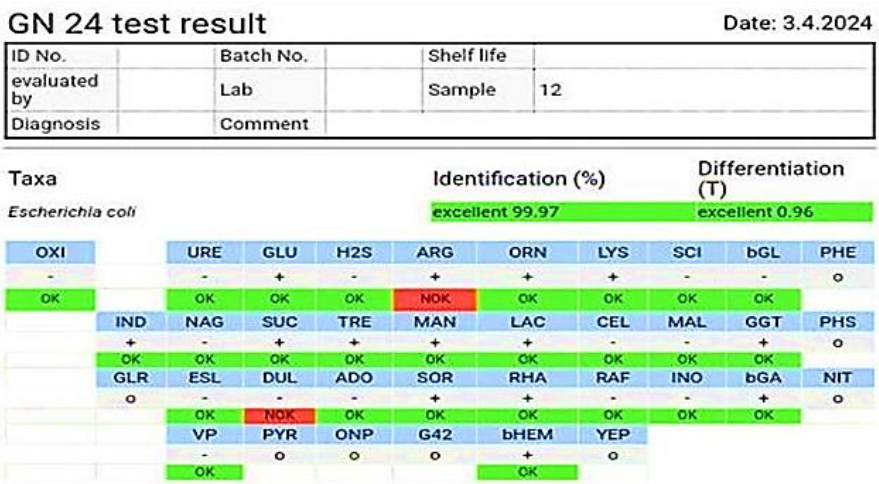

Table (3): *Different serotypes of selected E. coli isolates and their percentage*

***E. coli* serotype**	**n. serotype**	**percentage**
O78	6/30	20%
O1	6/30	20%
O18	3/30	10 %
O113	3 /30	10%
O119	3/30	10%
O103	2/30	6.6%
O111	2/30	6.6%
O25	2/30	6.6%
O26	2/30	6.6%
O104	1/30	3.3%

Table (4): *Antibiogram of 30 E. coli isolated from turkey***.**

	Antibiogram Phenotypic Pattern					
	Resistance				Sensitive	
	Resistant	Intermediate	Total	%*	No.	%*
Amoxicillin	23	1	24	80	6	20
Doxycycline	10	6	16	53.33	14	46.66
Chloramphenicol	23	2	25	83.3	5	16.66
Trimethoprim Sulphamethazole	11	0	11	36.66	19	63.33
Gentamycin	1	8	9	30	21	70
Colistin	0	0	0	0	30	100

Table (5) *Antibiogram of E. coli O78*

Code n. of isolate	CL	SXT	GEN	DO	AMC	C	MDR For
1	S	S	S	R	R	R	3
18	S	R	I	R	R	R	5
22	S	I	I	I	R	R	2
24	S	R	S	R	R	R	5
25	S	R	S	R	R	R	5

Table (6): *Identification of resistance genes in E. coli O78*

Code n.	blaTEM	floR	mph A	tetA(A)
1(1)	+	+	+	+
2(18)	+	+	+	+
3(22)	+	+	-	+
4(24)	-	+	+	+
5(25)	+	+	-	+

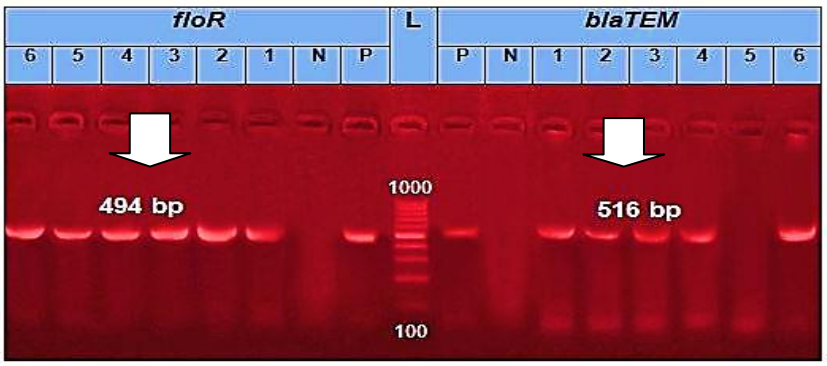

Figure (3): Agarose gel electrophoresis displaying the outcome of PCR for identification of BlaTEM and floR gene from 6 isolates.

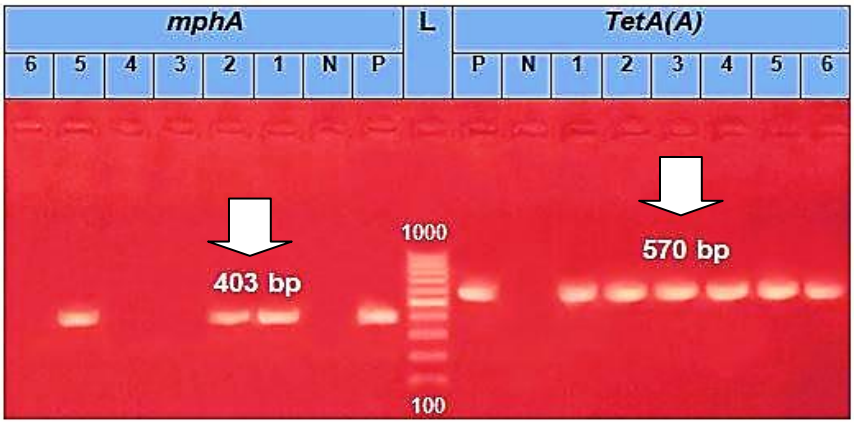

Figure (4): Agarose gel electrophoresis displaying the outcome of PCR for identification of TetA(A) and mphA gene from 6 isolates.

Discussion

Poultry and its products are associated with numerous foodborne infections in humans, posing a significant challenge due to the difficulty of implementing effective control measures. In addition, the existence of antibiotic-resistant bacteria in turkey meat and its products poses an ongoing threat to public health. Colibacillosis is a prominent foodborne illness that leads to diarrhea and can be fatal in both humans and animals; its detection in poultry farms serves as an indicator of environmental conditions ***(Bo et al., 2018)***. A rise in the proportion of β-lactamase-producing *E. coli* has been detected in both human populations and food samples, posing a considerable community health threat owing to the substantial presence of multidrug-resistant genes ***(Chong et al., 2011)***.

In this research, the prevalence rate of E. *coli* was 60%. This finding aligns with numerous previous instigations, including ***Altekruse et al. (2002),*** and ***Hoepers et al. (2018)*** with 89%,86.7%and 84%. Some researchers have reported even higher rates finding, like ***Tawyabur et al. (2020)*** with 100%. However, lower rates have been reported by ***Eid and Samir (2019)*** with 15%.

Ten different serotypes were identified within selected *E. coli* isolates, and the most prevalent one was *E. coli O78* and *E. coli O1* with 20%, while *O18, O113* and *O119* with 10%, *O103, O111, O25, O26* with 6.6% and *O104* with 3.3%. Serogroups *O78* is among the most common reported worldwide ***(D'Incau et al., 2006; Giovanardi et al., 2007, 2011; and Circella et al., 2009).*** The finding was higher than ***Osman et al. (2018)***, who found *O78* with 10% but the same in *O 119* with 10%

and lower in *O1* with 6.7% from the hatchlings and higher in *O78* from ***(AbdelRahman et al., 2020)*** which isolated from imported batches of turkey poults with 5.4%.

Antibiogram of *E. coli* revealed that 80% (24/30) were resistant to Amoxicillin+ clavulanic acid ,83.3%(25/30) for chloromphenicol, and 53.33%(16/30) to doxycyclin. The isolates were highly senstive for colistin with 100%. The high resistance to Amoxicillin clavulanic acid is likely due to β-lactamase production, with the blaTEM gene detected in 83.33% of isolates.These findings are consistent with ***Eid and Samir (2019)***, who reported 88.9% resistance to Amoxicillin clavulanic acid in addition to 94.4% of isolates were positive for blaTEM gene, and multiple other research reporting similar patterns ***(Guerra et al., 2003; Hoepers et al., 2018; Osman et al., 2018).***

E. coli isolates were highly resistant to Chloramphenicol, Erythromycin and Doxycycline with 74.19% 70.97% and 32.25% which regarded for presence of resistance genes doxycycline (TetA), erythromycin (MphA) and chloramphenicol (floR) detected with 100% in tested sample. This result matches with ***Tawyabur et al. (2020),*** who documented that all *E. coli* isolates were resistant to erythromycin and chloramphenicol while was 52.73% with tetracycline among them tetA was detected in 27 (27/29; 93.1%).

Conclusions

The present study *E. coli O78* isolated from turkey poults with the highest percent comparing to the other ten serotypes among thirty E. *coli* strains and detects phenotypic characterization of antimicrobial resistance on them. Also detect *Enterobactreace* specific resistance gene like blaTEM gene and TetA. It makes that indiscriminate use of antibiotics and addition of growth promoters in animal feed contributed to the emergence of resistance among *Enterobactrecea* and other bacteria.

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Ethics approval

Consent for sample collection from the turkeys was granted by the farm owners. All procedures followed the ARRIVE guidelines (https://arriveguidelines.org) and relevant regulations, and were authorized by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Suez Canal University (Ethical approval no.: SZUC 2018119).

Data availability

The article contains the data that underpins the outcomes of this study.

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الملخص العربي

تُسبب الإشريكية القولونية المُمرضة للطيور (APEC) عدوى جهازية أو موضعية ذات مسارات سريرية مُختلفة، مثل تسمم الدم، وداء الحويصلات الهوائية، والتهاب المصليات المتعددة، وورم القولون الحبيبي. يُسبب داء العصيات القولونية خسائر اقتصادية فادحة في قطاع الدواجن، نظرًا لضرورة السيطرة عليه عن طريق التسبب في تلوث الذبيحة، وانخفاض معدل تحويل العلف، ونفوق الدواجن. في هذه الدراسة، عُزلت 30 سلالة من الإشريكية القولونية من 50 ديك رومي مريض. أُجريت اختبارات حساسية جميع السلالات للمضادات الحيوية، وتم تحديد النمط المصلي لثلاثين سلالة مقاومة للأدوية المتعددة. لوحظت أعلى مقاومة ضد حمض الكلافولانيك الأموكسيسيلين، والكلورامفينيكول، والإريثروميسين، والدوكسيسيكلين. ولوحظت مقاومة متعددة للأدوية بنسبة 83.33% (30/25). من بين السلالات الثلاثين التي يمكن تحديد النمط المصلي لها، حُددت O78 وO1 على أنهما أكثر المجموعات المصلية انتشارًا بنسبة 20% (30/6) لكليهما، بينما بلغ معدل O18 وO113 و10% O119. في الوقت نفسه، بلغ معدل O103 وO111 وO25 و6.6% O26 كمجموعات مصلية نادرة. حُددت O78 كسلالة عالية المقاومة للمضادات الحيوية في سلالات الإشريكية القولونية المعزولة، ونمط مصلي سائد في السلالات المختارة. أُخضعت خمس سلالات من O78 للكشف عن 4 جينات مقاومة هي blaTEM وfloR وmph A وtetA (A). تحتوي جميع الخمسة على tetA (A) و floR بينما تحتوي أربع عزلات على blaTEM و mph A.