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Phylogenetic characterization and determination of antibiotic susceptibility of avian pathogenic *Escherichia coli* strains isolated from broiler visceral organs

Caracterización filogenética y determinación de la susceptibilidad a los antibióticos de cepas patógenas de *Escherichia coli* aviar aisladas de órganos viscerales de pollos de engorde

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ABSTRACT

The study aims to identify phylogenetic groups and antibiotic susceptibility of poultry *Escherichia coli* (APEC) isolates. *E. coli* was phenotypically and biochemically characterized. Isolates from 8/30 (26.66%) liver, 7/30 (23.33%) heart, and 4/30 (13.33%) spleen of 37-42 days old vaccinated broiler chickens were assessed. Then the *E. coli* isolates (19/90; 21.11%) were phylogrouped by quadruplex genotyping based on the presence or absence of *arpA*, *chuA*, *yjaA* genes, and TspE4.C2 DNA fragment.The majority of APEC strains belonged to phylogenetic group C, followed by groups A, E, and F. Phylogroup C was observed in the liver, phylogroup A in both liver and heart samples, phylogroup E in the heart and spleen, and phylogroup F in the liver. The highest antibiotic resistance was observed in Amoxicillin-Clavulanic acid and Ampicillin (100%) predominantly in groups A and E according to antibacterial susceptibility tests. Multiple antibiotic resistance (MDR) for APEC strains was also found at 68.42% (13/19). Of the 19 isolates tested, only 13 (68%) were susceptible to high levels of gentamicin. APEC strains belonging to phylogroups C, A, and E are of epidemiological importance for broilers. It would be beneficial to investigate new phylogroups by performing more detailed genotypic analyzes in APEC strains.

Key words: Broilers; molecular biology; *Escherichia coli*; phylogenetic; susceptibility

RESUMEN

El estudio tuvo como objetivo identificar los grupos filogenéticos y la susceptibilidad a los antibióticos de los aislados de *Escherichia coli* de aves de corral (APEC). *E. coli* se caracterizó fenotípica y bioquímicamente a partir de hígado, 8/30 (26,66 %); corazón, 7/30 (23,33 %) y bazo, 4/30 (13,33 %) de pollos de engorde de 37-42 días de edad vacunados. Luego, los aislados de *E. coli* (19/90; 21,11 %) se filoagruparon mediante genotipado cuádruple en función de la presencia o ausencia de los genes *arpA*, *chuA*, *yjaA* y el fragmento de ADN TspE4.C2. La mayoría de las cepas APEC pertenecían al grupo filogenético C, seguido de los grupos A, E y F. El filogrupo C se observó en el hígado, el filogrupo A en las muestras de hígado y corazón, el filogrupo E en el corazón y el bazo y el filogrupo F en el hígado. La mayor resistencia antibiótica se observó en amoxicilina-ácido clavulánico y ampicilina (100 %) predominantemente en los grupos A y E según pruebas de susceptibilidad antibacteriana. También se encontró resistencia múltiple a antibióticos (MDR) para las cepas APEC en 68,42 % (13/19). De los 19 aislamientos probados, solo 13 (68 %) fueron susceptibles a niveles altos de gentamicina. Las cepas APEC pertenecientes a los filogrupos C, A y E son de importancia epidemiológica para los pollos de engorde. Sería beneficioso investigar nuevos filogrupos realizando análisis genotípicos más detallados en las cepas APEC.

Palabras clave: Pollos de engorde; biología molecular, *Escherichia coli*; genética; microbiología

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INTRODUCTION

Escherichia coli is a pathogen implicated in intestinal and extraintestinal infections [40]. Intestinal pathogens include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [19]. Extra-intestinal pathogenic *E. coli* (ExPEC) are an important group of pathogenic *E. coli* causing systemic colibacillosis in poultry caused by avian pathogenic *E. coli* (APEC) and responsible for economic losses for the world's poultry industries [11]. Some of the strains of *E. coli* found in the lower gastrointestinal microbiota of poultry may spread viscerally and cause high morbidity and mortality (20%) in chicken (*Gallus gallus domesticus*) flocks [9]. It also causes loss of live weight (2%), decrease in feed efficiency (2.7%) and decrease in egg production (up to 20%) [20]. Avian pathogenic *E. coli* (APEC) causes mostly systemic colibacillosis disease resulting in significant local or regional economic losses in the poultry industry [10, 30]. Wild ducks (*Anas platyrhynchos*), geese (*Anser anser*), and wild fowls can be carriers of APEC and present a global threat to nutrition safety and poultry welfare [27]. APEC is common in all ages of chickens (9.52 to 36.73%) [18]. Besides the air sacs, strains may also infect the organs such as the spleen, peritoneum, pericardium, liver, yolk sac, pleura, and oviduct [12]. Some virulence genes found in APEC belonging to the phylogenetic group associated with extraintestinal pathogenic.

E. coli (ExPEC) have also been found in human ExPEC [13]. In addition, APEC group D has been reported to mostly belong to a phylogenetic group of ECOR. It has been reported that lesser virulent APECs and even avirulent commensal *E. coli* can be transmitted to immunocompromised avians [2]. Particularly, a positive relationship has been shown between neonatal meningitis-causing *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC). It indicates that APEC strains can be potential zoonotic agents [20, 25, 29]. APEC can share virulence factors with extraintestinal pathogenic *E. coli* associated with humans, and that case raises the possibility that APEC may play a role in some cases of human disease [29, 30]. It has also been stated that APEC and extraintestinal pathogenic *E. coli* (ExPEC) strains cause diseases in humans, and improperly cooked chicken meat can cause foodborne infections [22]. The uptaking of APEC plasmids by common *E. coli* strains increases virulence in infections (respiratory infections, septicaemia in poultry, dying of chicken embryos) [39, 41].

In addition, APEC can also be isolated from rodents (*Rattus*). Also, vertical transmission through contaminated eggs is also seen in infected chickens [18]. The zoonotic potential of APEC or other pathotypes isolated in chickens depends on the phenotypic characterization and appraisal of common serotypes isolated from infected chickens [34]. Understanding the structure of *E. coli* showed that strains that refer to different phylogroups may be related to the source of isolation [6]. Phylogenetic studies are important for understanding the *E. coli* population, strains, hosts, and the pathophysiology of the diseases [6]. The phylogenetic analysis has reported that *E. coli* consists of A, B1, B2, and D phylogenetic groups.

Also, it has been demonstrated that virulent extraintestinal *E. coli* strains are clustered in the main group B2 and to a secondary extent group D but conversely most of the commensal strains are associated with group A or group B1 [16, 40]. Although group C is closely related to group B1, it also includes strains of different genotypes. Group E was defined as a new group comprising previously unclassified strains. Group F was considered the very close group of B2 [5]. Antibiotics (Tetracyclines, Sulfonamides, and Aminoglycosides) are used to control colibacillus in chickens. However, increasing resistance to some groups of antibiotics (-Lactams, Colistin, and Carbapenems) also limits the use of antibiotics to control APEC infections in chickens [20].

The rapid spread of APEC to various visceral organs and the lead to septicemia characterized by lesions in multiple organs require more rational use of antimicrobials to reduce infection-related morbidity and mortality [22]. In addition, the potential of APEC to cause extraintestinal diseases in humans should also be considered [35]. Especially, currently, studies on antimicrobial-resistant ExPEC and ESBL generating *E. coli* are increasing [26]. This study aimed at the phylogenetic characterization and determination of the antibiotic susceptibility profiles of *E. coli* strains isolated from internal organs (liver, heart, and spleen) of broiler chickens.

MATERIALS AND METHODS

Sample collection

Ninety swab samples were collected from the internal organs (liver, heart and spleen) of 30 broiler chickens from different farms (average capacity 5,000-20,000 chickens) located in the Aegian and Western Black Sea Region in Turkey, between November 2021 and January 2022. Broiler chickens in these poultry houses are 39-45 days old and weigh 2,000-2,500 grams (g). All chickens have been vaccinated with commercial live vaccines such as Hipraclone® H120 (Spain), CEVAC® IBD-L (France), Nobilis® Ma5+Clone 30 and Nobilis® IB 4-91 (Netherland). Collected swab samples were brought to Aydin Adnan Menderes University, Veterinary Faculty, Microbiology Department diagnostic laboratory in a cold chain with Stuart transport medium.

Identification of *E. coli*

Once in the laboratory, swabs samples were immediately plated onto Columbia agar enriched with 5% sheep blood (Oxoid, Basingstoke, United Kingdom (UK)), then Urinary Tract Infection (UTI) Brilliance Clarity Chromogenic agar (Oxoid, Basingstoke, UK), and Brilliant Green agar (Oxoid, Basingstoke, UK), and were incubated aerobically at 37°C overnight. Identification was performed based on morphologic properties, and biochemical analyses [urease (-), catalase (-), Voges proskauer (-), indole (+), carbohydrate fermentation (+), methyl red (+), citrate (-)], then confirmed using the BD Phoenix™ 50 automatic identification appliance [Becton, Dickinson, and Company, Franklin Lakes, New Jersey, United States (USA)]. All samples were freezer-preserved in 50% glycerol brain heart infusion broth (Oxoid, Basingstoke, UK) at -20°C (Bosch, Series 4, Germany) until subsequent analysis.

DNA extraction

Total Deoxyribonucleic acid (DNA) extraction of APEC isolates which were identified with BD Phoenix™ 50 automatic identification appliance was carried out by using the Thermo Scientific™ Genomic DNA Purification Extraction Kit (Waltham, Massachusetts, USA), according to manufacturer instructions. Extracted DNA was quantified measured with the Nanodrop device (Maestrogen®, MN-913, Taiwan) and results were recorded. The extracted DNA were freezer-kept in cryotubes at -20°C (Bosch, Serie 4, Germany).

Polymerase chain reaction (PCR)

Phylogroups were determined by a PCR protocol developed and adapted by Clermont *et al.* [6]. The primer sequences used for the PCR reactions (HIMEDIA Prima Trio™ Thermal Cycler, India) are given in TABLE I.

TABLE I
List of primers used for phylotyping of avian pathogenic *E. coli* isolates[1]

PCR reaction	Primer	Target	Primer sequences	PCR product (bp)
Quadruplex	chuA.a1	*chuA*	5′-ATGGTACCGGACGAACCAAC-3′	288
	chuA.2		5′-TGCCGCCAGTACCAAAGACA-3′	
	yjaA.1b	*yjaA*	5′-CAAACGTGAAGTGTCAGGAG-3′	211
	yjaA.2b		5′-AATGCGTTCCTCAACCTGTG-3′	
	TspE4C2.1b	TspE4.C2	5′-CACTATTCGTAAGGTCATCC-3′	152
	TspE4C2.2b		5′-AGTTTATCGCTGCGGGTCGC-3′	
	AceK.f	*arpA*	5′-AACGCTATTCGCCAGCTTGC-3′	400
	ArpA1.r		5′-TCTCCCCATACCGTACGCTA-3′	
Group E	ArpAgpE.f	*arpA*	5′-GATTCCATCTTGTCAAAATATGCC-3′	301
	ArpAgpE.r		5′-GAAAAGAAAAAGAATTCCCAAGAG-3′	
Group C	trpAgpC.1	*trpA*	5′-AGTTTTATGCCCAGTGCGAG-3′	219
	trpAgpC.2		5′-TCTGCGCCGGTCACGCCC-3′	

[1]Reference (Clermont *et al.* [6])

First, a quadruplex PCR reaction was performed on avian pathogenic *E. coli* isolates, and then isolates in a particular phylogroup were determined according to the results obtained. In addition, PCR analyzes were performed on avian pathogenic *E. coli* isolates using group C- and group E-specific primers. The DNA from ATCC 25922 was used as a positive control for *E. coli*. Quadruplex and group C/E specific PCR reaction was examined at a total volume of 25 microliters (µL) including 2x Taq Mastermix (GenetBio®, South Korea) 12,5 µL; 10 pikomol (pmol) each forward and reverse primer 0,4 µL; 50-100 nanograms (ng) template DNA 2 µL and completed with nuclease-free water. Quadruplex PCR condition; initial denaturation 95°C during 5 minutes (min) 1 cycle; cyclic denaturation 95°C, 30 seconds (sec), annealing 59°C, 30 sec, elongation 72°C, 30 sec for 30 cycles; final elongation 72°C, 5 min one cycle. Group C/E specific PCR condition; initial denaturation 95°C, 5 min 1 cycle; cyclic denaturation 95°C, 30 sec, annealing 59°C, 30 sec for group C and 57°C, 30 sec for group E, elongation 72°C, 30 sec for 30 cycles; final elongation 72°C, 5 min one cycle. PCR products were examined on 1.5% agarose gel electrophoresis and visualized on the imaging system (Vilber-Lourmat™-Infinity VX2, France).

Phylogenetic grouping of APEC

Phylogroups of avian pathogenic *E. coli* isolates were determined that depending on the results of the quadruplex screening and the C, E clade PCRs [6].

Antibiotic susceptibility test of avian pathogenic *E. coli* isolates

Antimicrobial susceptibility testing (n=19) of APEC isolates was performed by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (MHA) at a 0.5 McFarland concentration [3]. Ampicillin [2 micrograms (2 µg)] (Oxoid, Hampshire, UK), Ciprofloxacin (5 µg), Enrofloxacin (5 µg), Tetracycline (30 µg), Gentamicin (10 µg), Erythromycin (10 µg), Sulfamethoxazole-Trimethoprim (25 µg) and Amoxicillin-Clavulanic acid (30 µg) discs were used for antibiogram tests. Inhibition zone diameters for *Enterobacteriaceae* were measured and evaluated according to clinical and laboratory standards institute (CLSI) [8].

RESULTS AND DISCUSSION

Phenotypic identification

From a total of 90 liver, spleen, and heart swab samples examined in this study. Nineteen (21.1%) APECs (liver: 8; heart: 7; spleen: 4) were identified, and they were confirmed with the BD Phoenix™ 50 automatic identification device (Becton, Dickinson, and Company, Franklin Lakes, New Jersey, USA). Of the 19 APEC isolates, twelve were from one organ, three were from liver and spleen organs, two were from a heart organ, and two were from liver and spleen organs. Different APEC strains isolated from the same sample were subjected to phylotyping separately.

Phylogenetic grouping of APEC isolates

As a result of phylotyping of 19 (21.1%), APEC isolates were identified by the quadruplex PCR method and 7 (36.8%) of them were typed as a single phylogroup. Two stage phylogroup typing was performed for the remaining 12 (63.2%) APEC isolates. Uniform phylogrouping was carried out according to the results obtained by PCR tests for groups E and C of these 12 APEC isolates. As a result, 7 (36.8%) of 19 APEC isolates were APEC phylogroup C, 5 (26.3%) were APEC phylogroup A, and 5 (26.3%) were APEC phylogroup E, and 2 (10.6%) were typed as APEC phylogroup F and their agarose gel electrophoresis micrograph were given in FIG 1. The distribution of phylogrouped 19 APEC isolates were shown in TABLE II.

Considering the distribution of phylogenetic groups of 19/90 (21.11%) APEC strains identified in this study, 5 APEC isolates were isolated to phylogroup A from 2 livers, 2 hearts, and 1 spleen. Also, 7 APEC isolates were isolated to phylogroup C from 4 livers, 2 hearts, 1 spleen, 5 APEC isolates were isolated to phylogroup E from 3 hearts, 2 spleens, and 2 APEC isolates were isolated to phylogroup F from 2 livers. There was not any *E. coli* strains isolated in the 71 (78.88%) samples.

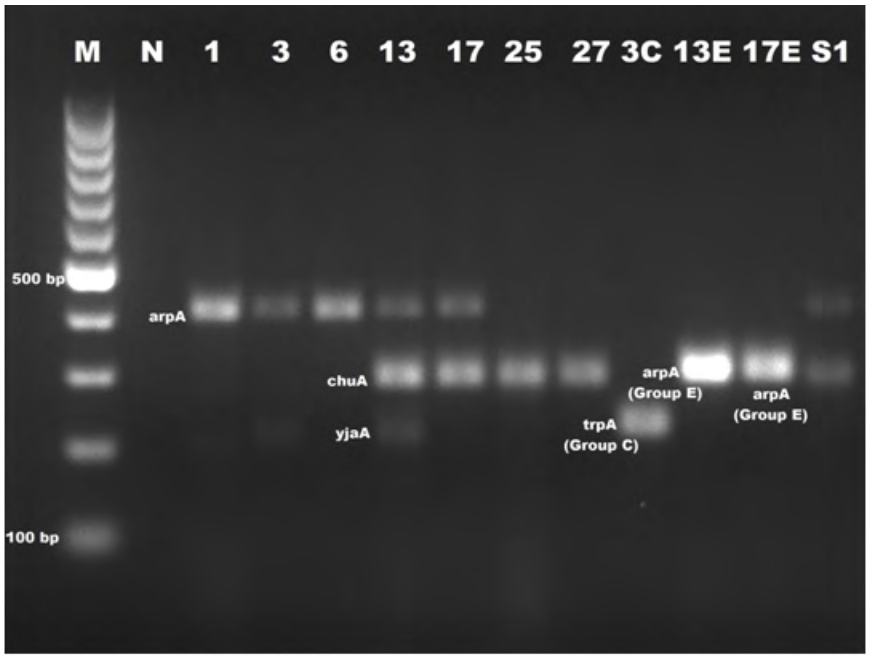

FIGURE 1. Phylogrouping of some APEC isolates. (M: molecular weight of marker) (100 bp); N: negative sample; 1: *arpA* (400 bp); 3/3C: *arpA* (400 bp) / *trpA* (Group C) (219 bp); 6: *arpA* (400 bp); 13/13E: *arpA* (400 bp) / *chuA* (288 bp) / *yjaA* (211 bp) / *arpA* (Group E) (301 bp); 17/17E: *arpA* (400 bp) / *arpA* (Group E) (301 bp); 25: *chuA* (288 bp); 27: *chuA* (288 bp); S1: *E. coli* standard strain (ATCC 25922)

TABLE II
Phylogroup distributions of APEC isolates according to quadruplex, group E, group C PCR results

Sample number of APEC isolates	Organs Sample	Quadruplex PCR				Pre-Phylogroup Results	Group E	Group C	Final Phylogroup Results
		arpA	*chuA*	*yjaA*	TspE4.C2		*arpA*	*trpA*	
1	Liver	+	-	-	-	A	-	-	A
3	Heart	+	-	+	-	A / C	-	+	C
6	Heart	+	-	-	-	A	-	-	A
10	Liver	+	-	+	-	A / C	-	+	C
12	Liver	+	-	+	-	A / C	-	+	C
13	Spleen	+	+	+	-	E / clade I	+	-	E
14L	Liver	+	-	-	-	A	-	-	A
14S1	Spleen	+	-	-	-	A	-	-	A
14S2	Spleen	+	-	+	-	A / C	-	+	C
17	Heart	+	+	-	-	D / E	+	-	E
21	Heart	+	+	-	-	D / E	+	-	E
22H1	Heart	+	-	-	-	A	-	-	A
22H2	Heart	+	-	+	-	A / C	-	+	C
23L	Liver	+	-	+	-	A / C	-	+	C
23S	Spleen	+	+	-	-	D / E	+	-	E
25	Liver	-	+	-	-	F	-	-	F
27	Liver	-	+	-	-	F	-	-	F
28	Liver	+	-	+	-	A / C	-	+	C
29	Heart	+	+	-	-	D / E	+	-	E

Antimicrobial susceptibility testing of *E. coli* isolates

The antibiogram tests were applied to (n=19) identified APEC isolates. APEC strains isolated from liver (1), heart (6) and spleen (1) were 100% resistant to all tested antibiotics. Strains isolated from liver (8), heart (7) and spleen (4) were also 100% resistant to Ampicillin and Amoxicllin-Clavulanic acid. In addition, following these percentages, other resistances rates were found as 87.5% for Ampicillin, Ciprofloxacin, Enrofloxacin, Erythromycin, Tetracycline, Sulfamethoxazole-Trimethoprim, and Amoxicllin-Clavulanic acid in the liver (4), heart (2), and spleen (1), 75% for Ampicillin, Ciprofloxacin, Enrofloxacin, Erythromycin, Tetracycline, and Amoxicllin-Clavulanic acid in the liver (1), and spleen (2), respectively. In contrast, antibiotic susceptibility rates were identified as 75% for Gentamicin in the liver (6), heart (3) and spleen (4), 12.5% both Ciprofloxacin and Enrofloxacin in the liver (1), and 25% for Sulfamethoxazole-Trimethoprim in the spleen (2) (TABLE III).

TABLE III
General results of antibiotic susceptibility tests of APEC isolates

No.	Isolate Name	AMP	CIP	ENR	E	GEN	TE	TMP-SXM	AMC
1.	L1	R	R	R	R	S	R	R	R
2.	L10	R	R	R	R	S	R	I	R
3.	L12	R	S	S	R	S	R	R	R
4.	L14	R	R	R	R	R	I	R	R
5.	L23	R	R	R	R	S	R	R	R
6.	L25	R	R	R	I	S	R	R	R
7.	L27	R	R	R	R	S	R	R	R
8.	L28	R	R	R	R	R	R	R	R
9.	H3	R	R	R	R	S	R	R	R
10.	H6	R	R	R	R	R	R	R	R
11.	H17	R	R	R	R	R	R	R	R
12.	H21	R	R	R	R	R	R	R	R
13.	H22A	R	R	R	R	R	R	R	R
14.	H22B	R	R	R	R	S	R	R	R
15.	H29	R	R	R	R	S	R	R	R
16.	S13	R	R	R	R	S	R	R	R
17.	S14A	R	R	R	R	S	R	S	R
18.	S14B	R	R	R	R	S	R	R	R
19.	S23	R	R	R	R	S	R	S	R

L, liver; H, heart; S, spleen; R, resistance; I, intermediate; S, susceptible; (AMP) Ampicillin 2 µg; (CIP) Ciprofloxacin 5 µg; (ENR) Enrofloxacin 5 µg; (E) Erythromycin 10 µg; (GEN) Gentamicin 10 µg; (TE) Tetracycline 30 µg; (TMP-SMX) Sulfamethoxazole-Trimethoprim 25 µg; (AMC) Amoxicillin-Clavulanic acid 30 µg

It was determined that APEC strains were 100% resistant to Amoxicillin-Clavulanic acid and Ampicillin, 94.7% to Ciprofloxacin, Erythromycin, Enrofloxacin, Tetracycline, and 84.22% to Sulfamethaxazole-Trimethprim, respectively. Generally, APEC strains showed 84% or more resistance to 7 antibiotics used in the research. Especially isolates (n=8/19) obtained from heart samples showed resistance to all antibiotics. Multiple antibiotic resistance (MDR) APEC strains were also found as 68.42% (13/19). Of the 19 isolates tested, only 13 (68%) were susceptible to high levels of Gentamicin. Multidrug resistance profiles of APEC isolates were shown in TABLE IV.

TABLE IV
Multidrug resistances profiles of APEC isolates

No. of Antibiotics	Antibiotic Profiles	Name of Isolates	No. of Isolates	Origin of Isolates
8	AMP, CIP, ENR, E, GEN, TE, TMP-SXM, AMC	L28, H6, H17, H21, H22A	5	Liver(1), Heart(4)
7	AMP, CIP, ENR, E, TE, TMP-SXM, AMC	H3, H22B, H2, S13, S14B	5	Heart (3), Spleen(2)
7	AMP, CIP, ENR, E, TE, TMP-SXM, AMC	L1, L23, L27	3	Liver
7	AMP, CIP, ENR, E, GEN, TMP-SXM, AMC	L14	1	Liver
6	AMP, CIP, ENR, E, TE, AMC	L10, S14A, S2	3	Liver(1), Spleen(2)
6	AMP, CIP, ENR, TE, TMP-SXM, AMC	L25	1	Liver
5	AMP, E, TE, TMP-SXM, AMC	L12	1	Liver

L, liver; H, heart; S, spleen; R, resistance; I, intermediate; S, susceptible; (AMP) Ampicillin 2 µg; (CIP) Ciprofloxacin 5 µg; (ENR) Enrofloxacin 5 µg; (E) Erythromycin 10 µg; (GEN) Gentamicin 10 µg; (TE) Tetracycline 30 µg; (TMP-SMX) Sulfamethoxazole-Trimethoprim 25 µg; (AMC) Amoxicillin-Clavulanic acid 30 µg

Avian pathogenic *E. coli* causes major localized or systemic losses in the poultry industry characterized by colisepticemia. It also concerns public health as potential zoonotic agents [29, 30]. *E. coli* is at least eight phylogenetic groups and is divided into three clusters: phylogroups B2, G, and F, phylogroups A, B1, C, and E, and phylogroup D (phylogroup D, the closest group to *E. coli* origin). Virulence genes are mostly associated with phylogroups D and B2 [5, 27].

APEC an opportunistic pathogen can cause secondary infections in visceral organs such as Newcastle disease, and Mycoplasmosis [15]. Phylogroup C is considered a different strain group closely related to phylogroup B1. It is stated that group F consists of strains very close to the B2 phylogroup. The inclusion of *arpA* has it possible to identify the misidentified strain D phylogroup (*chuA*+, *yjaA*-, *TspE4.C2*-), which should have previously belonged to F.

These genes out of the strains, due to they are present in all *E. coli* strains rather than the strains which belong to B2 and F phylogroups, should be differentiated [7]. In this study, extended quadruple PCR was preferred as the phylogroup assignment method because it provides advantages in detecting strains belonging to C, E, F, and clade I phylogroups, although a small part of *E. coli* strains cannot be included in a phylogroup and has disadvantages such as variable gene content [6]. Commensal *E. coli* belongs to phylogenetic group A. Groups D, most closely related to *E. coli*, consist of several evolutionary lineages considered "virulent clones" [17].

APEC strains were isolated and identified from liver, heart, and spleen organ swaps collected from 2,000-2,500 g of 39-45 days old broiler chickens. In this study, *E. coli* isolates (19/90; 21.11%) obtained from chicken viscera showed similarity to previously reported studies [34, 37]. Logue *et al***.** [24] has been reported the distribution of phylogenetic groups of 452 APEC strains isolated from poultry according to the quadruplex PCR method (Clermont *et al*. [5]).

The rates of the phylogroups was A (10.17%), C (27.65%), D (5.08%), E (3.31%), and F (19.26%), respectively. Ungrouped strains were reported as 0.22%. In other studies, the D phylogenetic group was specified as commonly found strains [4, 21]. As a result of this study, the A, C, and E groups were found to be higher than the results they obtained. The number of strains not included in any phylogroup was determined at a higher rate (APEC; 78.88%). D and B2 are Groups of *E. coli* responsible for extraintestinal diseases.

Phylogenetically, Group E is closely related to Group D (including O157: H7), and also Group F is closely related to B2 [38]. In this study, group F was found to be 10.52% among APEC strains. Subsequently, according to the study by Coura *et al.* [9], higher results were observed for Groups A (2.66%), F (3.33%), and E (12.00%), but similar results were observed for Groups D (0.00%).

E. coli isolates in phylogroups A and D were reported to have spread from breeders to broilers, and strains of phylogroup B2 and D were accepted as extraintestinal pathogenic *E. coli* [2, 28, 33]. In this study, phylotyping of strain A was made, but no phylotyping of extraintestinal Group D was observed. However, the closely related Group E was isolated. Phylogroups D and A are the dominant phylogroup in APEC types [5, 6, 11, 41]. The obtained results support the knowledge that A phylogroup is the most common strains obtained from broiler chickens [9]. However, unlike Coura *et al***.** [9] the obtained findings highlight that phylogroups C, E, and F can also be isolated from broilers.

APEC isolates were tested with 8 antimicrobial agents to examine antibiotic susceptibility. Resistance was observed to Ampicillin and Amoxicillin-Clavulanic acid (19/19; 100%), followed by Ciprofloxacin, Enrofloxacin, Erythromycin, and Tetracycline (18/19; 94,7%), and finally to Sulfamethoxazole-Trimethoprim (16/19; 84,21%) and Gentamicin (6/19; 31,58%). In addition, susceptibility was observed for Gentamicin (13/19; 68.42%), Sulfamethoxazole-Trimethoprim (2/19; 10.52%), Ciprofloxacin, Enrofloxacin, and Erythromycin (1/19; 5.26%) in isolates.

Various studies have been conducted on resistance and susceptibility. It has been reported that APEC isolates were resistant to Sulfamethoxazole-Trimethoprim (95.5%), Amoxicillin (93.3%), Ampicillin (89.6%), Amoxicillin-Clavulanate (79%), Gentamicin (68.8%), Ciprofloxacin (47.9%), and Tetracycline (45%) [14, 36]. The obtained results confirm the resistance but draw attention to the high rates of resistance, especially to Amoxicillin-Clavulanate, Ampicillin, Tetracycline, and Ciprofloxacin. It has been reported that discontinuing the non-therapeutic use of antibiotics (such as Tetracyclines) prescribed to promote growth in poultry led to a significant reduction in resistant bacteria, which can be seen especially in poultry [23]. Therefore, it is important not to use unnecessary antibiotics to prevent the development of resistance.

The high APEC resistance to Tetracycline has been reported [31, 32]. The present findings also confirm this evaluation. In a study, the highest percentage of sensitivity for Enrofloxacin (53.85%) and Gentamicin (46.15%) has been reported [1], but in this study, the sensitivity was determined only at the Gentamicin level, and, Enrofloxacin was not found to be very effective. The high levels of resistance observed for the antibiotic classes in this study suggest that they are widely used in the avian industry. Besides, it was pointed out that the origin of APEC strains may be poultry material and may be important in infecting other animals by being present in the environment.

CONCLUSIONS

This study demonstrated that APEC strains could be isolated from edible organs. Also, strains may spread from poultry residuals to the environment and may transmit to other animals in this way. *Escherichia coli* poses a serious threat to human health and food safety.

In the present study, phylogroup C was the most common group. Phylogroups A and E were generally isolated from the heart and spleen, while phylogroups C and F were detected from liver and heart samples. For this reason, ensuring hygiene in the poultry houses is important both in terms of poultry health and providing safe food to people.

The indiscriminate use of antibiotics in animal production may contribute to the resistant strains of APEC strains. Because of its zoonotic importance, routine laboratory research targeting APEC virulence genes for the early detection of avian colibacillosis may be beneficial in preventing unnecessary antibiotic use and resistance. Also, It would be beneficial to investigate new phylogroups by performing more detailed genotypic analyzes in APEC strains.

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BIBLIOGRAPHIC REFERENCES

[1] AWAD, A.M.; EL-SHALL, N.A.; KHALIL, D.S.; EL-HACK, M.E.A.; SWELUM, A.A.; MAHMOUD, A.H.; EBAID, H.; KOMANY, A.; SAMMOUR, R.H.; SEDEIK, M.E. Incidence, pathotyping, and antibiotic susceptibility of avian pathogenic *Escherichia coli* among diseased broiler chicks. **Pathog.** 9: 114. 2020.

[2] BARBIERI, N.L.; OLIVEIRA, A.L.D.; TEJKOWSKI, T.M.; PAVANELO, D.B.; ROCHA, D.A.; MATTER, L.B.; HORN, F. Genotypes and pathogenicity of cellulitis isolates reveal traits that modulate APEC virulence. **PLoS One.** 8: e72322. 2013.

[3] BAUER, A.W.; KIRBY, W.M.; SHERRIS, J.C.; TURCK, M. Antibiotic susceptibility testing by a standardized single disk method. **Am. J. Clin. Pathol.** 45: 493–496. 1966.

[4] CHAUDHURI, R.R.; HENDERSON, I.R. The evolution of the *Escherichia coli* phylogeny. **Infect. Genet. Evol.** 12: 214–226. 2012.

[5] CLERMONT, O.; BONACORSI, S.; BINGEN, E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. **App. Environ. Microbiol.** 66: 4555–4558. 2000.

[6] CLERMONT, O.; CHRISTENSON, J.K.; DENAMUR, E.; GORDON, D.M. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. **Environ. Microbiol. Rep.** 5: 58–65. 2013.

[7] CLERMONT, O.; OLIER, M.; HOEDE, C.; DIANCOURT, L.; BRISSE, S.; KEROUDEAN, M. Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. **Infect. Genet. Evol.** 1: 654–662. 2011.

[8] CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). Performance standards for antimicrobial susceptibility testing. 27th. Ed. M100, Wayne, USA. Pp 32–39**.** 2017.

[9] COURA, F.M.; DINIZ, D.S.; SILVA, M.X.; MUSSI, J.M.S.; BARBOSA, S.M.; LAGE, A.P.; HEINEMANN, M.B. Phylogenetic group of *Escherichia coli* isolates from broilers in Brazilian poultry slaughterhouse. **Sci. World J.** 2017:1–7. 2017.

[10] CUMMINS, M.L., REID, C.J.; CHOWDHURY, P.R.; BUSHELL, R.N.; ESBERT, N.; TIVENDALE, K.A.; DJORDJEVIC, S.P. Whole genome sequence analysis of Australian avian pathogenic *Escherichia coli* that carry the class 1 integrase gene. **Microb. Genom.** 5: e000250. 2019.

[11] EWERS, C.; JANßEN, T.; KIEßLING, S.; PHILIPP, H.C.; WIELER, L.H. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. **Vet. Microbiol.** 104: 91–101. 2004.

[12] GUABIRABA, R.; SCHOULER, C. Avian colibacillosis: still many black holes. **FEMS Microbiol. Lett.** 362: fnv118. 2015.

[13] GYLES, C.L.; FAIRBROTHER, J.M. *Escherichia coli*. In: **Pathogenesis of Bacterial Infections in Animals.** 4th. Ed. Blackwell Publishing, New Jersey, USA. Pp 267–379. 2010.

[14] IBRAHIM, R.A.; CRYER, T.L.; LAFI, S.Q.; BASHA, E.A; GOOD, L.; TARAZI, Y.H. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. **BMC Vet. Res.** 15: 159. 2019.

[15] IEVY, S.; ISLAM, M.; SOBUR, M.; TALUKDER, M.; RAHMAN, M.; KHAN, M.F.R. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. **Microorganis.** 8: 1021. 2020.

[16] IRANPOUR, D.; HASSANPOUR, M.; ANSARI, H.; TAJBAKHSH, S.; KHAMISIPOUR, G.; NAJAFİ, A. Phylogenetic groups of *Escherichia coli* strains from patients with urinary tract infection in Iran based on the new Clermont phylotyping method. **Biomed. Res. Int.** 2015: 846219. 2015.

[17] JOHNSON, J.R.; STELL, A.L. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. **J. Infect. Dis.** 181: 261–272. 2000.

[18] KABIR, S.M. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns, **Int. J. Environ. Res.Public Health**. 7: 89–114. 2010.

[19] KAPER, J.; NATARO, J.; MOBLEY, H. Pathogenic *Escherichia coli*. **Nat. Rev. Microbiol.** 2:123–140. 2004.

[20] KATHAYAT, D.; LOKESH, D.; RANJIT, S.; RAJASHEKARA, G. Avian Pathogenic *Escherichia coli* (APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. **Pathog.** 10: 467. 2021.

[21] KOGA, V.L.; RODRIGUES, G.R.; SCANDORIEIRO, S.; VESPERO, E.C.; OBA, A.; DE BRITO, B.G.; DE BRITO, K.C.; NAKAZATO, G.; KOBAYASHI, R.K. Evaluation of the Antibiotic Resistance and Virulence of *Escherichia coli* Strains Isolated from Chicken Carcasses in 2007 and 2013 from Paraná, Brazil. **Foodborne Pathog. Dis.** 12: 479–485. 2015.

[22] KRISHNEGOWDA, D.N., SINGH, B.R.; MARIAPPAN, A.K.; MUNUSWAMY, P.; SINGH, K.P.; SAMINATHAN, M.; REDDY, M.R. Molecular epidemiological studies on avian pathogenic *Escherichia coli* associated with septicemia in chickens in India. **Microb. Pathog.** 162: 105313. 2022.

[23] LEVY, S. Reduced antibiotic use in livestock: how Denmark tackled resistance. **Environ. Health Perspect.** 122: A160–A165. 2014.

[24] LOGUE, C.M.; WANNEMUEHLER, Y.; NICHOLSON, B.A.; DOETKOTT, C.; BARBIERI, N.L.; NOLAN, L.K. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. **Front. Microbiol.** 8: 283. 2017.

[25] MALUTA, R.P.; LOGUE, C.M.; CASAS, M.R.T.; MENG, T.; GUASTALLI, E.A.L.; ROJAS, T.C.G.; DA SILVEIRA, W.D. Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) *Escherichia coli* isolated in Brazil. **PLoS One.** 9: e105016. 2014.

[26] MANGES, A.R. *Escherichia coli* and urinary tract infections: the role of poultry-meat. **Clin. Microbiol. Infect.** 22: 122–129. 2016.

[27] MEHAT, J.W.; VAN VLIET, A.H.; LA RAGIONE, R.M. The avian pathogenic *Escherichia coli* (APEC) pathotype is comprised of multiple distinct, independent genotypes. **Avian Pathol.** 50: 402–416. 2021.

[28] MICENKOVÁ, L.; BOSÁK, J.; ŠTAUDOVÁ, B.; KOHOUTOVÁ, D.; ČEJKOVÁ, D.; WOZNICOVÁ, V.; VRBA, M.; ŠEVČIKOVÁ, A.; BUREŠ, J.; ŠMAJS, D. Microcin determinants are associated with B2 phylogroup of human fecal *Escherichia coli* isolates. **Microbiol.** 5: 490–498. 2016.

[29] NAKAZATO, G.; CAMPOS, T.A.D.; STEHLING, E.G.; BROCCHI, M.; SİLVEİRA, W.D.D. Virulence factors of avian pathogenic *Escherichia coli* (APEC). **Pesqui. Vet. Bras.** 29: 479–486. 2009.

[30] NOLAN, L.K.; BARNES, H.J.; VAILLANCOURT, J.P.; ABDUL-AZIZ, T.; LOGUE, C.M. Colibacillosis. **Dis. Poult.** 13: 751–805. 2019.

[31] OSMAN, K.M.; KAPPELL, A.D.; ELHADIDY, M.; EL MOUGY, F.; EL-GHANY, W.A.A.; ORABI, A.; YOUSEF, H.M. Poultry hatcheries as potential reservoirs for antimicrobial-resistant *Escherichia coli*: a risk to public health and food safetyi. **Sci. Rep.** 8: 1–14. 2018.

[32] OZAWA, M.; HARADA, K.; KOJIMA, A.; ASAI, T.; SAMESHIMA, T. Antimicrobial susceptibilities, serogroups, and molecular characterization of avian pathogenic *Escherichia coli* isolates in Japan. **Avian Dis.** 52: 392–397. 2008.

[33] PASQUALI, F.; LUCCHI, A.; BRAGGIO, S.; GIOVANARDI, D.; FRANCHINI, A.; STONFER, M.; MANFREDA, G. Genetic diversity of *Escherichia coli* isolates of animal and environmental origins from an integrated poultry production chain. **Vet. Microbiol.** 178: 230–237. 2015.

[34] RAMADAN, H.; AWAD, A.; ATEYA, A. Detection of Phenotypes, Virulence Genes and Phylotypes of Avian Pathogenic and Human Diarrheagenic *Escherichia coli* in Egypt. **JIDC.** 10: 584–591. 2016.

[35] RODRIGUEZ-SIEK, K.E.; GIDDINGS, C.W.; DOETKOTT, C.; JOHNSON, T.J.; FAKHR, M.K.; NOLAN, L.K. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. **Microbiol.** 151: 2097–2110. 2005.

[36] RUŽAUSKAS, M.; ŠIUGŽDINIENĖ, R.; KRIKŠTOLAITIS, R.; VIRGAILIS, M.; ZIENIUS, D. Prevalence and antimicrobial resistance of *E. coli* isolated from chicken liver sold in retail markets. **Vet. ir Zoot.** .52: 67–72. 2010.

[37] SARBA, E.J.; KELBESA, K.A.; BAYU, M.D.; GEBREMEDHIN, E.Z.; BORENA, B. M.; TESHALE, A. Identification and antimicrobial susceptibility profile of *Escherichia coli* isolated from backyard chicken in and around ambo. Central Ethiopia. **BMC Vet. Res.** 15: 85. 2019.

[38] SAROWSKA, J.; FUTOMA-KOLOCH, B.; JAMA-KMIECIK, A.; FREJ-MADRZAK, M.; KSIAZCZYK, M.; BUGLA-PLOSKONSKA G.; CHOROSZY-KROL, I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. **Gut Pathog.** 11: 10. 2019.

[39] SKYBERG, J.A.; JOHNSON, T.J.; JOHNSON, J.R.; CLABOTS, C.; LOGUE, C.M.; NOLAN, L.K. Acquisition of avian pathogenic *Escherichia coli* plasmids by a commensal *E. coli* isolate enhances its abilities to kill chicken embryos, grow in human urine, and colonize the murine kidney. **Infect. Immun.** 74: 6287–6292. 2006.

[40] TENAILLON, O.; SKURNIK, D.; PICARD, B.; DENAMUR, E. The population genetics of commensal *Escherichia coli*. **Nat. Rev. Microbiol.** 8: 207–217.2010.

[41] WANG, X.M.; LIAO, X.P.; ZHANG, W.J.; JIANG, H.X.; SUN, J.; ZHANG, M. J.; LIU, Y.H. Prevalence of serogroups, virulence genotypes, antimicrobial resistance, and phylogenetic background of avian pathogenic *Escherichia coli* in south of China. **Foodborne Pathog. Dis.** 7: 1099–1106**.**