Original Article

Phylogenetic Analysis of Attaching and Effacing *E. coli* Strains Isolated From Pet Birds in Iran

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ABSTRACT

Background: Enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) are categorized as attaching and effacing *E. coli* (AEEC) due to their *eae* gene. One of the essential causes of diarrhea in humans is AEEC, which affects birds, too, thereby being considered a zoonotic pathogen.

Objectives: Our study aimed to determine AEEC and evaluate its antibiotic resistance and phylogroups.

Methods: A total of 200 fecal samples were collected from pet birds referred to the Veterinary Medicine Hospital, University of Tehran. PCR methods were used to detect AEEC using *uspA*, *eae*, *bfpA*, *stx1*, and *stx2* gene-specific primers. The antimicrobial susceptibility of the recovered isolates was determined by the agar disk diffusion and MIC methods. Their phylogroups were analyzed based on Clermont phylotyping methods.

Results: Of 200 samples, we isolated 26 (13%) *E. coli* strains, 9 harbor *eae* genes. None of the ease-positive samples possessed the *bfpA* gene, but 4 had *stx2*, and 5 had *stx1* and *stx2* genes. Phylogenetic analysis identified the phylogenetic groups of all AEEC isolated strains but 2 (duck and cockatiel). Detected phylogroups include four B2 and three D. Based on our results, 7 out of 9 AEEC isolated strains showed multi-drug resistance.

Conclusion: The discovery of common phylogroups of AEEC in pet birds (a common companion animal in Iran with intimate contact with their owners, especially children) and humans, as well as their resistance to a wide range of antibiotics used in human medicine, verifies AEEC as a serious public health threat.

Keywords: Attaching and effacing *E. coli* (AEEC), *Escherichia coli (E. coli)*, Phylogrouping, Shiga toxin, Shiga toxin-producing *E. coli* (STEC)

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Introduction

Various agents, such as viruses, bacteria, and parasites, cause diarrhea in humans. Of bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) ranks among the most important causes (Gomes et al., 2016).

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, non-sporulating, and facultative anaerobic bacterium of *Escherichia* and the family Enterobacteriaceae (Shahrani et al., 2014). Diarrheagenic strains of *E. coli* are divided into four main categories: Enterotoxigenic *E. coli* (ETEC), which causes diarrhea by increasing intestinal secretion; enteroinvasive *E. coli* (EIEC) that invades intestinal cells and causes diarrhea (like *Shigella* spp.); enterohemorrhagic *E. coli* (EHEC) that causes intestinal disease by intimate adherence to the intestinal epithelium and the development of Shiga-like toxin (SLT); and enteropathogenic *E. coli* (EPEC) that is characterized by its intimate adherence to the intestinal epithelial cell membranes (Gomes et al., 2016).

E. coli is the most prevalent opportunistic enterobacteria in captive animals and is associated with systemic disease in birds. Airsacculitis and sepsis are frequently caused by Avian pathogenic *Escherichia coli* (APEC) pathotypes, classified as extraintestinal pathogenic *E. coli* (ExPEC). Although the etiology of *E. coli*-induced enteritis in birds is unknown, diarrheagenic strains could pose a public health danger. Shiga toxin-producing *E. coli* (STEC) and EPEC represent two of at least 6 pathotypes of human diarrheagenic *E. coli* (EPEC, EHEC, ETEC, EAEC [Enteroaggregative *E. coli*], EIEC, and DAEC [diffusely adherent *E. coli*]) that infect birds and are zoonotic pathogens (Godambe et al., 2017; Kaper et al., 2004). *E. coli* strains (EHEC and EPEC) that generate characteristic attaching and effacing (A/E) lesions in the intestinal mucosa are classified as attaching and effacing *E. coli* (AEEC). A/E lesions are characterized by the intimate adhesion of the bacterium to the epithelial cell membrane and the effacement of the enterocyte's microvilli (Gomes et al., 2016).

STECs are also one of the most commonly transferred infections through food. They can cause food poisoning with moderate (like diarrhea) to severe clinical manifestations (such as hemolytic uremic syndrome [HUS] and hemorrhagic colitis [HC]) and, in some cases, death. The O157: H7 serotype, described as the most crucial serotype of this strain, is most typically linked to HC and HUS. Foodborne bacterial epidemics have been observed due to consuming undercooked or raw meat contaminated with STEC strains (Zarei et al., 2019).

STEC pathogenicity is influenced by various parameters, including their ability to generate A/E lesions and produce one or more cytotoxins from the Shiga toxin (Stx) family. Shiga toxins are among the primary virulence factors of STEC, created by their encoded bacteriophage genes *stx1* and *stx2*. Two prominent families of *stxs* are *stx1* and *stx2*. *Stx2* appears more toxic than *stx1* and has been related to HC and HUS (Gomes et al., 2016). As previously noted, several STEC strains can develop A/E lesions by employing fimbriae for colonization. The interaction between Tir (translocated intimin receptor) and intimin, along with transducing signals between bacterial and host cells, creates an intimate adherence between the bacterium and the epithelial cell membrane and pedestal formation beneath adherent bacteria. It can destroy intestinal epithelial-cell microvilli and consequent A/E lesions (Gomes et al., 2016).

EC/STEC pathogenicity is a multi-stage process, so in addition to producing toxins and forming A/E lesions, other factors, such as various toxins and adhesion factors, contribute to their virulence (Rivas et al., 2016).

Pathogenic *E. coli* most often causes avian disease in birds, classified as ExPEC pathotype. This pathotype has virulence traits like adhesion and invasion, the ability to produce toxins and protectins, and the potential to exhibit iron uptake pathways that match their extraintestinal lifestyle. Although most APECs are extraintestinal, as aforementioned, some possess common properties with the intestinal *E. coli* pathotypes (Johnson et al., 2022).

EPECs are the other strains of AEEC, known because of their ability to form A/E lesions but their inability to produce Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins (Nataro & Kaper, 1998). A/E lesions are associated with intimin, a 94-kDa protein encoded by the *eae* gene, present in LEE, a ~35-kb pathogenicity island (PAI). Intimin is classified into several distinct subtypes represented by the Greek letters: α (alpha) through ζ (zeta). β (beta) intimin is the most common subtype in APEC (Gomes et al., 2016).

EPEC strains are subclassified into tEPEC and aEPEC based on the presence or absence of *bfpA* genes, respectively. This gene is found in the EPEC adherence factor (EAF) plasmid (pEAF), a large virulence plasmid that encodes bundle-forming pilus (BFP), a type IV fimbriae. BFP plays an essential role in the initial adherence to tEPEC (Gomes et al., 2016).

EPEC and STEC can be detected and differentiated by various approaches, like PCR assay that uses primers targeting the *eae* and *stx* genes. The isolates that test positive for the *eae* gene are categorized as AEEC. The presence of the *stx* gene distinguishes between STEC and EPEC. Strains harboring this gene are classified as STEC; otherwise, they are categorized as EPEC. To differentiate tEPEC from aEPEC, PCR should check all *eae*-positive and stx-negative *E. coli* strains for the presence of the *bfpA* gene and or the EAF plasmid. However, some current PCR assays are not suitable methods for the detection of tEPEC strains because multiple alleles of *bfpA* have been identified (Blank et al., 2000; Franke, et al., 1994; Gunzburg et al., 1995).

E. coli is divided into phylogroups: A, B1, B2, C, D, E, F, and clade I. Another phylogroup called G was discovered by Clermont et al. (2019). According to their findings, 70% of phylogroup G strains encodes one or more resistance genes, implying that antimicrobial resistance factors are widespread. Multidrug resistance (MDR) was also discovered in 53% of these isolates. They suggested that the *ybgD* gene (567 bp) is unique to the G phylogroup and may be used to distinguish this phylogroup from others. The necessity of knowing the phylogroup of isolated strains arises from the fact that phylogroup B2 has higher virulence than phylogroup D, and extraintestinal pathogenic strains are more likely to belong to group B2 than group D. Group A includes the majority of commensal strains.

Multiple studies employing isolated *E. coli* strains from human feces have discovered the phylogroups A, B1, B2, C, D, E, F, and clade I (Clermont et al., 2013; Watson et al., 2021). On the other hand, *E. coli* strains isolated from Psittaciformes fecal samples belonged to similar phylogroups, such as F and clade I (Gioia-Di Chiacchio et al., 2016). This finding should alarm us as a zoonotic health threat to the general public.

As previously indicated, there have been various human investigations into diarrheagenic agents. Diarrheagenic *E. coli* strains, particularly EPEC and STEC, are among the most dangerous ones. Psittaciformes have been identified as a reservoir for diarrheagenic *E. coli* strains, significant pathogens associated with child mortality in tropical countries. On the other hand, the role of other avian species as reservoirs for these pathogens has remained unclear. Previous research in Iran has revealed that diarrheagenic *E. coli* strains such as STEC and EPEC are the most common causes of diarrhea, particularly in children (Abbasi et al., 2013). Most *E. coli* strains are harmless in the intestines and seldom infect healthy people. However, healthy and immunocompromised individuals might develop diarrhea or extraintestinal disorders due to several pathogenic strains. In addition to being a serious public health issue, diarrheal diseases (especially caused by EPEC and STEC) are a leading cause of morbidity and mortality in newborns and young children, particularly in developing countries (Gomes et al., 2016).

This issue emphasizes the need to investigate the presence of AEEC in companion birds, which are possible reservoirs for the bacterium and come into close contact with humans, especially children. Furthermore, some findings suggest that these strains may increase budgerigar mortality, highlighting their economic value in ornamental bird breeding (Seeley et al., 2014). In this study, we investigated the prevalence of AEEC in fecal samples collected from pet birds. Furthermore, the isolated strains were examined for their antibiotic resistance profiles and phylogroup.

Materials and Methods

Our study collected 200 fecal samples from 22 avian species, especially *Psittaciformes* and *Passeriformes*, which are housed as pets (Table 1). There was variation in the age and sex of the examined birds. The swab samples were first cultured in LB (Luria Bertani) broth. After 18 hours of incubation at 37°C, the samples were plated on MacConkey agar and re-incubated at 37°C for 18 hours. All possible *E. coli* isolates were stored in LB broth containing 15% glycerol at -20°C (for a short time until further processing).

For DNA extraction, the boiling method was used (Zahraei Salehi et al., 2007). For specific detection of *E. coli* strains, the isolated samples were investigated for the presence of the *uspA* (universal stress protein A) gene based on Chen & Griffiths' study (1998). In the next step, *uspA* gene-positive samples were examined for *eae*, *bfpA*, *stx1*, and *stx2* virulence genes (Paton & Paton, 1998; Scaletsky et al., 2002).

Clermont et al.'s techniques (Clermont et al., 2013; Clermont et al., 2019) were utilized for phylogenetic analysis of isolated AEEC strains. Based on these approaches, *E. coli* strains are assigned to one of the phylogroups A, B1, B2, C, D, E, F, clade I, and G.

For all PCR procedures, the positive control was the O157:H7 strain that had already been isolated, and the negative control was sterile water. Each step's PCR products were separated on 1.5% agarose gels (Yektata-

jhiz, Tehran, Iran) in TBE (Tris Base, Boric Acid, EDTA, pH 8, 0.5M), dyed with safe stain (SinaClon BioScience, Tehran, Iran) and viewed under ultraviolet light illumination (Kiagen, Tehran, Iran).

Table 2 presents the primer sequences used in our investigation to detect the *uspA* gene, *eae, stx1, stx2*, and *bfpA* virulence genes, the genes utilized for phylogroup analysis, and their references.

Isolated AEEC strains were examined for their antibiotic resistance characteristics in the final stage. For this stage, we used disk diffusion (DD) and minimum inhibitory concentration (MIC) methods based on the Clinical Laboratory Standard Institute (CLSI, 2020) standard. Briefly, for DD, we inoculated a suspension of overnight growth bacteria in LB broth with turbidity equivalent to a 0.5 McFarland standard on the un-supplemented Mueller-Hinton (MH) agar using cotton swabs. After 15 minutes, the antibiotic disks (18 antibiotics used for this method are listed in Table 3) (Padtan Teb®, Iran) were placed on MH agar and finally incubated at 35±1˚C for 24 hours. Then, the results are read based on CLSI guidance. For MIC, the microdilution method was used in sterile round-bottomed 96-well microplates, and different microplates were used for each strain of AEEC. Serial dilution (64 through 0.12 µL/mL) was prepared for each antibacterial agent (9 antibiotics (Rooyan Darou, Tehran, Iran) were used for this method mentioned in Table 3), and LB broth growth bacteria with turbidity equivalent to 0.5 McFarland scale was added to each well. The results were read after 24 hours of incubation at 37˚C.

Results

E. coli was isolated from 26 out of 200(13%) birds by investigating the *uspA* gene. The white-eared bulbul had the highest percentage of isolated *E. coli* (60%), followed by duck (37.5%), canary (20%), mynah, and rose-ringed parakeet (18.2%), budgerigar (14.2%), African grey parrot and lovebirds (12.5%), and cockatiel (10.1%) (Table 1). Among 26 isolated *E. coli* strains, 9 AEEC (3 cockatiel, 2 mynah, 2 white-eared bulbul, 1 rose-ringed parakeet, and 1 duck) were detected based on the presence of the *eae* gene. All AEEC isolates were classified as STEC based on the absence of the *bfpA* gene and the presence of *stx1* and or *stx2* genes. Only 5 isolated STEC strains possessed *stx1* and *stx2* genes, and others only had *stx2* virulence genes.

We applied Clermont et al.'s (2019) upgraded phylogroup approach to analyze our STEC isolates' phylogroups. Seven out of 9 AEEC strains showed a determined phylogroup, including 4 phylogroup B2 (cockatiel, mynah, rose-ringed parakeet, and white-eared bulbul) and 3 phylogroup D (cockatiel, mynah, and white-eared bulbul). Phylogroups of cockatiel and duck were non-typeable (Table 1).

According to our findings, most STEC strains (7 out of 9) were resistant to at least three antibacterial drug classes. Multidrug resistance (MDR) was observed in our isolated STEC strains (Table 3).

Discussion

Some strains of *E. coli* are commensal in mammals' and some avian species' gastrointestinal tracts, whereas others can cause intestinal and extraintestinal disorders with a wide range of clinical symptoms (Gomes et al., 2016).

STEC is one of the diarrheagenic *E. coli* pathotypes. This pathotype is recognized for its ability to produce Shiga toxin, a type of cytotoxin that inhibits protein synthesis in eukaryotic cells. It is also important because of its zoonotic potential. Poultry and cattle are the principal reservoirs of STEC pathotypes, particularly the O157:H7 serotype (Kim et al., 2020).

The most prevalent form of infection transmission is through food, although infection through human contact with infected companion animals such as dogs, cats, and birds is also possible (Kim et al., 2020).

Twenty-two different bird species belonging to 5 orders (Psittaciformes, Passeriformes, Anseriformes, Apodiformes, and Accipitriformes) were examined in our study, including cockatiel (89), mynah (22), lovebirds (16), rose-ringed parakeet (11), green-cheeked parakeet (8), duck (8), African grey parrot (8), budgerigar (7), white-eared Bulbul (5), monk parakeet (5), canary (5), finch (5), Old World sparrows (2), conures (1), grass parakeets (1), Eclectus parrot (1), Amazon parrot (1), Iraq babbler (1), wrens (1), starling (1), common swift (1), and common buzzard (1). It turned out that 34% of isolated *E. coli* belonged to A/E *E. coli*. Positive-*eae* gene strains were found in 40% of the white-eared bulbuls (2 out of 5), 12.5% of the ducks (1 out of 8), 9% of the mynahs (2 out of 22), 9% of the rose-ringed parakeets (1 out of 11), and 3.3% of the cockatiels (33 out of 89).

All isolated strains were classified as Shiga Toxin-producing *E. coli* (STEC), which should be considered because of their zoonotic potential. Different studies have

Table 1. The list of investigated avian species, along with detected virulence genes (*eae, stx1, and stx2)* and phylogroups

No.	Order	Bird Species	No. of Samples	*E. coli* +	*eae* +	*stx1* +	*stx2* +	*bfpA* +	Phylogroup
1	Psittaciformes	Cockatiel	89	9	3	3	3	-	D, (-), B2
2		Lovebirds	16	2	-	-	-	-	-
3		Rose-ringed Parakeet	11	2	1	-	1	-	B2
4		Green-cheeked parakeet	8	-	-	-	-	-	-
5		African grey parrot	8	1	-	-	-	-	-
6		Budgerigar	7	1	-	-	-	-	-
7		Monk parakeet	5	-	-	-	-	-	-
8		Conures	1	-	-	-	-	-	-
9		Grass parakeets	1	-	-	-	-	-	-
10		Eclectus parrot	1	-	-	-	-	-	-
11		Amazon parrot	1	-	-	-	-	-	-
12	Passeriformes	Mynah	22	4	2	1	2	-	D, B2
13		White-eared Bulbul	5	3	2	1	2	-	D, B2
14		Canary	5	1	-	-	-	-	-
15		Finch	5	-	-	-	-	-	-
16		Old world sparrows	2	-	-	-	-	-	-
17		Iraq babbler	1	-	-	-	-	-	-
18		Wrens	1	-	-	-	-	-	-
19		Starling	1	-	-	-	-	-	-
20	Anseriformes	Duck	8	3	1	-	1	-	(-)
21	Apodiformes	Common swift	1	-	-	-	-	-	-
22	Accipitriformes	Common buzzard	1	-	-	-	-	-	-
Total	-	-	200	26(13%)	9(4.5%)	5(2.5%)	9(4.5%)	0(0%)	-

been carried out on STEC in birds in Iran. Although most of them focused on the foodborne aspect of this agent, another study by Koochakzadeh et al. (2015) indicated a low percentage of STEC isolation in wild and companion birds. In total, 2.28% (5 out of 219) *E. coli* strains were detected, of which 4(80%) and 1(20%) were categorized as STEC and EPEC, respectively. However, in our study, among 26(13%) isolated *E. coli* strains, the *eae* gene was detected in 9(34.6%) strains, all identified as STEC. Both studies demonstrated a high frequency of STEC among *eae*-positive strains with an increase in their frequency (1.8% [4 out of 219] to 4.5% [9 out of 200]), about 2.5 times more than the previous study, in which the *stx2* gene was the main isolated virulence gene. As already described, the toxicity of *stx2* is more than *stx1* and, in most cases, is associated with HC and

Table 2. The list of primers and their sequences used in this study

PCR Reaction	Primer ID	Target Gene	DNA Primers (5'–3')	Ampl Size (bp)	Reference
-	-	*uspA*	F. CCGATACGCTGCCAATCAGT R. ACGCAGACCGTAGGCCAGAT	884	Chen & Griffiths., 1998
-	-	*Eae*	F. GACCCGGCACAAGCATAAGC R. CCACCTGCAGCAACAAGAGG	384	Paton & Paton, 1998
-	-	*bfpA*	F. CACCGTTACCGCAGGTGTGA R. GTTGCCGCTTCAGCAGGAGT	450	Scaletsky et al., 2002
-	-	*stx1*	F. ATAAATCGCCATTCGTTGACTAC R. AGAACGCCCACTGAG ATCATC	180	Paton & Paton, 1998
-	-	*stx2*	F. GGCACTGTCTCTCTGAAACTGCTC R. TCGCCAGTTATCTGACATTCTG	255	
Quadruplex	AceK.f ArpA1.r	*arpA*	F. AACGCTATTCGCCAGCTTGC R. TCTCCCCATACCGTACGCTA	400	Clermont et al., 2013
	chuA.1b chuA.2	*ChuA*	F. ATGGTACCGGACGAACCAAC R. TGCCGCCAGTACCAAAGACA	288	
	yjaA.1b yjaA.2b	*yjaA*	F. CAAACGTGAAGTGTCAGGAG R. AATGCGTTCCTCAACCTGTG	211	
	TspE4C2.1b TspE4C2.2b	*TspE4C2*	F. CACTATTCGTAAGGTCATCC R. AGTTTATCGCTGCGGGTCGC	152	
Group E	ArpAgpE.f ArpAgpE.r	*arpA*	F. GATTCCATCTTGTCAAAATATGCC R. GAAAAGAAAAAGAATTCCCAAGAG	301	
Group C	trpAgpC.1 trpAgpC.2	*trpA*	F. AGTTTTATGCCCAGTGCGAG R. TCTGCGCCGGTCACGCCC	219	
Internal control	trpBA.f trpBA.r	*trpA*	F. CGGCGATAAAGACATCTTCAC R. GCAACGCGGCCTGGCGGAAG	489	
Group G	ybgD.f ybgD.r	*ybgD*	F. GTTGACTAARCGYAGGTCGA R. KATGYDGCYGATKAAGGATC	567	Clermont et al., 2019

HUS. So, further study is needed to investigate the severity of their pathogenicity as a zoonotic pathogen. According to a study conducted by Zahraei Salehi et al. (2007) among 12 APEC isolates that belonged to the most common serotypes in Iran, the *stx2* gene was detected in 75% (9 out of 12) of isolates, while only 8.3% (1 out of 12) possessed both *stx1* and *stx2*. Furthermore, 16.66% of isolates possessed the *eae* gene. This study's results demonstrated that the *stx2* virulence factor may be widespread among APEC in Iran, which agrees with our study because we detected *stx2* in all isolated STEC strains. Zarei et al. (2019) investigated the prevalence of STEC in 257 raw chicken meat samples collected. In total, they found *E. coli* in 36% (93 out of 257) of samples, with STEC, EPEC, and AEEC accounting for 38.7% (36 out of 93), 7.5% (7 out of 93), and 12.9% (12 out of 93), respectively. Also, a high rate of resistance to some antibiotic agents like nalidixic acid (91.4%), tetracycline (89.8%), ampicillin (82.8%), and sulfamethoxazole-trimethoprim (71%) was detected by using the DD method. That finding was similar to the antibiotic resistance profile in our study. In Iran, the majority of STEC studies focused on food-borne transmission methods, such as chicken meat (Momtaz et al., 2013), raw milk (Momtaz et al., 2012; Mohammadi, 2013; Brenjchi, 2011), minced meat (Kazemi Galougahi, 2012), carcasses of sheep (Jafareyan-Sedigh, 2011), hamburger (Jamshidi, 2012; Kargar, 2013), water and vegetables (Shah Illi, 2010), lettuce (Mazaheri, 2014), beef meat (Sami, 2007), and the like (Hooman, et al., 2020b).

Gioia-Di Chiacchio et al. (2016) collected 171 fecal samples from Psittaciformes (67 cockatiels, 59 budgerigars, and 45 love birds). They identified 42(24.5%) *E. coli* strains, among which 19.4% (8 out of 42) were positive for the *eae* and *stx2* genes. Isolated STEC strains were detected with percentages of 8.47% (5 out of 59) in budgerigars, 4.47% (3 out of 67) in cockatiels, and 0% (0 out of 45) in

Table 3. The results of antibiotic resistance profile of isolated strains by Using DD and MIC

No.	Antimicrobial Class or Subclass	Agents Included: Generic Names	118		122		135		156		158		162		165		170		171	
			DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
1	β-Lactam/β-lactamase inhibitors	Amoxiclav	R	R	R	R	R	R	R	R	I	S	R	R	S	S	R	R	R	R
2	Penicillins	Ampicillin	R	R	R	R	R	R	R	R	S	S	R	I	S	S	S	S	S	S
3	Cephems (parenteral)	Cefotaxime	R	-	I	-	I	-	I	-	I	-	R	-	S	-	I	-	I	-
4		Ceftriaxone	S	S	R	I	S	S	I	S	S	S	I	S	I	S	S	S	S	S
5		Cefixime	I	-	I	-	I	-	I	-	I	-	R	-	S	-	R	-	I	-
6		Ceftazidime	I	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S
7	Fluoroquinolone	Ciprofloxacin	R	R	R	R	R	R	R	I	S	S	I	I	I	R	S	S	S	I
8		Levofloxacin	R	-	R	-	R	-	R	-	S	-	S	-	S	-	S	-	S	-
9		Norfloxacin	R	-	I	-	S	-	I	-	S	-	S	-	S	-	S	-	S	-
10		Ofloxacin	R	-	I	-	I	-	I	-	S	-	S	-	S	-	S	-	S	-
11	Phenicols	Chloramphenicol	R	-	R	-	R	-	R	-	S	-	S	-	R	-	R	-	S	-
12	Tetracyclines	Doxycycline	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	S
13		Tetracycline	R	R	R	R	R	R	R	R	S	S	S	S	R	R	S	S	S	I
14	Fosfomycins	Fosfomycin	S	-	S	-	S	-	S	-	S	-	S	-	S	-	S	-	S	-
15	Aminoglycosides	Gentamicin	S	S	R	I	S	S	S	S	S	S	S	S	R	I	S	S	S	S
16		Streptomycin	S	-	R	-	R	-	R	-	I	-	R	-	R	-	R	-	I	-
17	Folate synthesis inhibitor	Sulfamethoxazole–trimethoprim	R	R	R	R	R	R	R	R	I	R	S	S	S	I	S	I	S	S
18	Quinolone	Nalidixic acid	R	-	R	-	R	-	R	-	S	-	S	-	S	-	S	-	S	-

love birds. The majority of STEC isolates in their study belong to budgerigars. In contrast, in our research, the white-eared bulbul has the highest percentage (40%) of STEC infection, and no STEC was detected from budgerigars. Aparecida et al. (2017) studied the outbreak of AEEC by investigating the fecal samples of 516 bird species belonging to 10 different orders, including Accipitriformes (14), Anseriformes (80), Columbiformes (72), Falconiformes (46), Galliformes (50), Passeriformes (88), Pelecaniformes (9), Piciformes (10), Psittaciformes (99), and Strigiformes (48). About 77.7% (401 out of 516) *E. coli* strains were detected from the collected fecal samples. Multiplex PCR for detecting *eae*, *bfpA*, *stx1*, and *stx2* genes was done on *E. coli* isolates. They found that 23(5.7%), 16(3.9%), and 3(0.7%) out of 401 *E. coli* strains were positive for the presence of *eae*, *bfpA*, and *stx2* genes, respectively. None of the strains were positive for the *stx1* gene. Based on their results, the prevalence rates of STEC, tEPEC, and aEPEC are significant and should be considered for their zoonotic potential. The orders in which AEEC was discovered were Psittaciformes (13 out of 99), Strigiformes (1 out of 48), and Columbiformes (9 out of 72). At the same time, our study revealed that only 4 out of 150(2.6%) birds associated with Psittaciformes had AEEC. On the other hand, the rate of isolated STEC strains in our study was significantly (about 6.4 times) higher than their results.

Until now, more than 100 STEC strains have been identified. Although the O157:H7 serotype is the most common cause of human infection, other non-O157 STEC serogroups such as O26, O111, O103, and O145 have been isolated from involved humans (Nataro & Kaper, 1998). Serogrouping of isolated strains was not part of our goals because we investigated the occurrence of AEEC in pet birds. However, we recommend that this topic should be examined in the future.

Antibiotic resistance was found in isolated *E. coli* bacteria at high levels, including tetracycline, sulfamethoxazole, ampicillin, streptomycin, and carbenicillin (Kang et al., 2005). MDR was observed in 77.8% (7 out of 9) of the isolated strains in this study because these strains demonstrated resistance to three or more classes of examined antibiotics (Gioia-Di Chiacchio et al. (2016).

A high prevalence of resistance was found for amoxiclav (7 out of 9), chloramphenicol (6 out of 9), and streptomycin (6 out of 9). However, all of the recovered isolates were sensitive to ceftazidime and fosfomycin. It is worth mentioning that most examined antibiotic agents are used in human medicine, and this rate of resistance to antibiotics is an alarm to pay more attention to this pathogen.

The prevalence of STEC strains in children or adults fecal samples has been investigated in several studies. A review article found these results: 5 out of 395(1.3%) from children and adults with diarrhea (Taghadosi, 2018), 36 out of 117(30.7%) from humans with HIV or thalassemia (Alizade, 2017), 34 out of 685(4.9%) children with diarrhea (Mohammadi-Sardo, 2017), 11 out of 147(7.4%) from stool samples of *E.coli*-positive strains collected from a human with diarrhea (Zarringhalam, 2016),15 out of 285(5.3%) in children <2 years old with diarrhea (Abbasi, 2014), 7 out of 615(1.1%) from children <5 years old with diarrhea (Kargar& Homayoon 2009). The evidence reveals a significant frequency of STEC in gastroenteritis patients (Hooman et al., 2020a). The discovery of comparable phylogroups in humans and animals (such as birds) suggests that this pathogen could be transmitted from animal to human.

Clermont phylotyping methods (Clermont et al., 2013; Clermont et al., 2019) were utilized to determine isolated STEC phylogenetic groups. Four strains (cockatiel, rose-ringed parakeet, mynah, and white-eared bulbul) were categorized as group B2. In contrast, three (cockatiel, mynah, and white-eared bulbul) as phylogroup D. Two strains, one from a duck and the other from a cockatiel, were not typable. In a study by Gioia-Di Chiacchio et al. (2016), phylogroup B2 was also found in AEEC strains isolated from Psittaciformes. F and clade I were also identified in their research.

Shiga-toxin-producing *E. coli* strains are one of the most important diarrheagenic *E. coli* with zoonotic potential and should be considered a serious risk to public health. In addition to the isolation of these strains from companion birds (4.5%), as a popular pet in our country, the significant isolation of STEC from patients suffering from gastroenteritis highlights more attention to this agent as a risk to public health, particularly children and those people who suffer from immunosuppression diseases. Furthermore, the high resistance to a wide range of antibiotics (such as amoxiclav, chloramphenicol, and streptomycin) used in human medicine and the discovery of common phylogroups of STEC in pet birds and humans (phylogroups B2 and D that belong to virulent lineages of *E. coli*) make STEC a considerable public health threat.

Conclusion

In conclusion, this study reveals a significant prevalence of Shiga toxin-producing *E. coli* (STEC) in various bird species, particularly in pet birds. This study highlights the zoonotic potential of these avian STEC strains,

supported by their occurrence in patients with gastroenteritis. The observed antibiotic resistance, especially to commonly used human antibiotics, raises concerns for public health. Shared phylogroups between pet birds and humans emphasize the need for broader surveillance and control measures to address the potential risk of STEC transmission. Overall, this study underscores the importance of considering avian STEC as a serious public health threat and warrants further research to understand and mitigate its impact

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Investigation, writing–original draft preparation: Mina Abbasi; Study design, data analysis, review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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مطالعه پژوهشی

تحلیل فیلوژنتیکی سویه‌های اشرشیاکلی اتصال و آسیب (AEEC) جداشده از پرندگان زینتی در ایران

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چکیده

زمینه مطالعه: انتروپاتوژنیک اشرشیاکلی (EPEC) و اشرشیاکلی‌های تولیدکننده شیگاتوکسین (STEC) به‌دلیل دارا بودن ژن eae جزء اشرشیاکلی‌های اتصال و آسیب دسته‌بندی می‌شوند. AEEC یکی از عوامل مهم اسهال در انسان‌ها هستند که می‌توانند پرندگان را هم درگیر کنند و باید به‌عنوان یک عامل بیماری‌زای قابل انتقال بین انسان و حیوانات مورد توجه قرار گیرند.

هدف: بررسی حضور سویه‌های AEEC در پرندگان زینتی، بررسی میزان مقاومت آنتی‌بیوتیکی آن‌ها و تعیین گروه‌های فیلوژنتیکی سویه‌های جداشده، به‌عنوان هدف این مطالعه در نظر گرفته شد.

روش کار: درمجموع ۲۰۰ نمونه مدفوعی از پرندگان زینتی ارجاعی به بخش پرندگان زینتی بیمارستان دامپزشکی دانشگاه تهران جمع‌آوری شدند. نمونه‌های مشکوک به اشرشیاکلی ازنظر حضور ژن‌های stx2، stx1، bfpA، eae، uspAمورد بررسی قرار گرفتند. در مرحله بعد گروه‌های فیلوژنتیکی سویه‌های AEEC جداشده تعیین شدند. در مرحله آخر مقاومت آنتی‌بیوتیکی سویه‌های یادشده با کمک دو روش آگار دیسک دیفیوژن و حداقل غلظت مهاری مورد بررسی قرار گرفتند.

نتایج: به‌طورکلی، ۲۶ سویه اشرشیاکلی (۱۳ درصد) از نمونه‌های جمع‌آوری‌شده جداسازی شدند. از این میان، ۹ نمونه دارای ژن eae بودند، و هیچ‌یک از آن‌ها ژن bfpA نداشتند. ۴ نمونه تنها دارای ژن حدت stx2 و ۵ نمونه دارای هر دو ژن حدت stx1 و stx2 بودند. در گروه فیلوژنتیکی ۷ سویه از ۹ سویه AEEC قابل تشخیص بود که شامل ۴ مورد گروه فیلوژنتیکی B2 و ۳ مورد گروه فیلوژنتیکی D بودند. در این مطالعه مقاومت آنتی‌بیوتکی چندگانه (MDR) در ۷۷/۷ درصد از نمونه‌ها مشاهده شد.

نتیجه‌گیری نهایی: شناسایی سویه‌های AEEC در پرندگان زینتی (به‌عنوان یکی از رایج‌ترین حیوانات خانگی در ایران که دارای ارتباط نزدیک با صاحب خود به‌ویژه کودکان است) دارای گروه فیلوژنتیکی مشترک با سویه‌های جداشده از انسان است و همچنین مقاومت آن‌ها به طیف وسیعی از آنتی‌بیوتیک‌های مورداستفاده در طب انسانی، بیانگر اهمیت مطالعه AEECها به‌عنوان تهدیدی جدی برای سلامت عمومی می‌باشد.

کلیدواژه‌ها: اشرشیاکلی، اشرشیاکلی اتصال و آسیب، اشرشیاکلی تولیدکننده شیگاتوکسین، تحلیل فیلوژنتیکی، شیگاتوکسین

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