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Phylogenetic Analysis of Attaching and Effacing *E. coli* (AEEC) Strains Isolated from Pet Birds in Iran

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Running head: Phylogrouping of Attaching and Effacing *E. Coli* in Pet Birds

Abstract

Background: Due to possessing the *eae* gene, enteropathogenic *E. coli* (EPEC) and shigatoxin-producing *E. coli* (STEC) are categorized as attaching and effacing *E. coli*. AEEC is one of the important causes of diarrhea in humans that affects birds and may be considered as a zoonotic pathogen.

Objectives: Our study aimed to determine attaching and effacing *E. coli,* evaluate their antibiotic resistance, as well as investigate their phylogroups.

Methods: 200 fecal samples were collected from pet birds referred to the veterinary medicine hospital, University of Tehran. PCR methods were used for the detection of AEEC by using *usp*A, *eae*, *bfp*A, *stx*1, and *stx*2 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.

Result: We isolated 26 (13%) *E. coli* strains, nine of which harbor *eae* genes. None of the *eae* positive samples possessed the *bfp*A gene, but four of them had *stx*2, and five had both *stx*1 and *stx*2. Phylogenetic analysis identified the phylogenetic groups of all AEEC isolated strains but two (duck and cockatiel). Detected phylogroups include four B2 and three D. Based on our results, seven out of nine AEEC isolated strains showed multi-drug resistance (MDR).

Conclusions: 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, establishes AEEC as a serious public health threat.

Keywords: AEEC, *E. coli*, Phylogrouping, Shiga toxin, STEC

Introduction

Diarrhea in humans is caused by a wide variety of agents such as viruses, bacteria, and parasites. Among bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is one of the most important causes of diarrhea (Gomes *et al.,* 2016).

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, non-sporulating, and facultative anaerobic bacterium of the genus *Escherichia* and the family Enterobacteriaceae (Shahrani *et al.*, 2014) Diarrheagenic strains of *E. coli* are divided into four main categories: 1) Enterotoxigenic *E. coli* (ETEC) that cause diarrhea due to enhanced intestinal secretion, 2) Enteroinvasive *E. coli* (EIEC) that invade intestinal cells and cause diarrhea like Shigella *spp.*, 3) Enterohemorrhagic *E. coli* (EHEC) produce intestinal disease by intimate adherence to the intestinal epithelium and the development of Shiga-like toxin (SLT), and 4) Enteropathogenic *E. coli* (EPEC) are characterized by intimate adherence between the bacterium and intestinal epithelial cell membranes (Gomes *et al*., 2016).

E. coli can be considered 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, which are classified as Extraintestinal pathogenic *E. coli* (ExPEC) pathotypes. Although the etiology of *E. coli*-induced enteritis in birds is unknown, the presence

of diarrheagenic strains could pose a public health danger. Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) represent two of at least six pathotypes of human diarrheagenic *E. coli* (EPEC, EHEC, ETEC, EAEC (Enteroaggregative *E. coli*), EIEC, and DAEC (Diffusely adherent *E. coli*)) that infect birds and may be considered as 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 by 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 as well as moderate (like diarrhea) to severe clinical manifestations (such as Hemolytic Uremic Syndrome (HUS) and hemorrhagic colitis (HC)) and, in some cases, ultimate death. The O157: H7 serotype, which is described as the most important serotype of this strain, is most typically linked to hemorrhagic colitis and hemolytic uremic syndrome. Foodborne bacterial epidemics have been observed as a result of consuming undercooked or raw meat contaminated with STEC strains (Zarei *et al.,* 2019).

STEC pathogenicity is influenced by a variety of parameters, including their ability to generate AE lesions and produce one or more cytotoxins from the Shiga toxin (Stx) family. Shiga toxins are one of the primary virulence factors of STEC, which are produced by their encoded bacteriophage genes *stx*1 and *stx*2. Two major families of stxs are stx1 and stx2. Stx2 appears to be more toxic than stx1 and has been related to HC and HUS (Gomes *et al.,* 2016). As previously noted, several STEC strains can develop AE

lesions. For this purpose, they use fimbriae for colonization. Then, by causing intimate adherence between the bacterium and the epithelial cell membrane and pedestal formation beneath adherent bacteria, which is mediated by the interaction between Tir (translocated intimin receptor) and intimin, and also transducing signals between bacterial and host cells, can destruct intestinal epithelial-cell microvilli and consequently lead to AE lesions (Gomes *et al*., 2016).

In total, EHEC/STEC pathogenicity is a multi-stage process, so in addition to producing toxins and forming AE lesions, other factors such as toxins of various types and also different adhesion factors contribute to their virulence (Rivas *et al.*, 2016).

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

EPECs are the other strains of AEEC that are known because of their ability to form AE lesions and their incapacity to produce Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins (Nataro & Kaper, 1998). AE lesions are associated with intimin, a 94-kDa protein encoded by the *eae* gene, which is 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 sub-classified into tEPEC and aEPEC based on the presence or absence of *bfp*A gen 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. An important role for BFP in the initial adherence to tEPEC was described (Gomes *et al.,* 2016).

EPEC and STEC can be detected and differentiated using a variety of approaches. One of these is a 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 should be studied to distinguish between STEC and EPEC. Strains harboring this gene are classified as STEC; otherwise, they should be categorized as EPEC. To differentiate tEPEC from aEPEC, PCR should be used to check all *eae*-positive and *stx*-negative *E. coli* strains for the presence of the *bfp*A gene and/or the EAF plasmid. However, some current PCR assays are not valuable methods for the defined detection of tEPEC strains, because multiple alleles of *bfp*A have been identified (Blank *et al*., 2000; Franke,' *et al*., 1994; Gunzburg *et al*., 1995).

E. coli is divided into various phylogroups, including 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 encoded 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 *ybg*D 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 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 have been found to belong to similar phylogroups such as F and clade I (Chiacchio *et al.,* 2016). This should be considered a zoonotic threat to the general public's health.

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. Psittaciformes have been identified as a reservoir for diarrheagenic *E. coli* strains, which are major pathogens associated with child mortality in tropical countries. On the other hand, the role of other avian species as reservoirs for these pathogens remains 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). The majority of *E. coli* strains are harmless in the intestines and seldom infect healthy people. However, both healthy and immunocompromised individuals might develop diarrhea or extraintestinal disorders as a result of several pathogenic strains. In addition to being a serious public health issue, diarrheal diseases (especially 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 topic 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 play a role in increasing 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, As well as the isolated strains were examined for their antibiotic resistance profiles, and also their phylogroup were analyzed.

Materials and Methods

In our study, we worked on 200 fecal samples collected from 22 different 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 reincubated at 37°C for 18 hours. All of the 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 *usp*A (universal stress protein A) gene based on Chen & Griffiths' study, (1998). In the next step, *usp*A gene-positive samples were examined for the presence of *eae*, *bfp*A, *stx*1, and *stx*2 virulence genes (Paton & Paton, 1998; Scaletsky *et al.,* 2002).

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

For all PCR procedures, the positive control was an 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 percent agarose gels (Yektatajhiz, 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 *usp*A gene, *eae*, *stx*1, *stx*2, and *bfp*A virulence genes, as well as 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 by 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°C ± 1 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

The results of investigating the *usp*A gene indicated that *E. coli* was isolated from 26 out of 200 (13 %) of the birds. 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 are classified as STEC based on the absence of the *bfp*A gene and the presence of *stx*1 and/or *stx*2 genes. Only 5 isolated STEC strains possessed both *stx*1 and *stx*2 genes, and other strains only had *stx*2 virulence genes.

We applied Clermont *et al.*'s (2019) upgraded phylogroup approach to analyze our STEC isolates' phylogroups. 7 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 two of them (Cockatiel and Duck) were non-typeable (Table 1).

According to our findings, the majority of STEC strains (7/9) 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 can also be transmitted to humans via contact with infected companion animals such as dogs, cats, and birds (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 AE *E. coli*. Positive *eae* gene strains were found in 40% of the White-eared bulbuls (2/5),

12.5% of the Ducks (1/8), 9% of the Mynahs (2/22), 9% of the Rose-ringed parakeets (1/11), and 3.3% of the Cockatiels (33/89).

All isolated strains were classified as Shiga Toxin-producing *E. coli* (STEC) that should be considered because of their zoonotic potential. Different studies have been carried out on STEC in birds in Iran. Although most of them focused on the foodborne aspect of this agent, another study was done by Koochakzadeh *et al.* (2015) indicated a low percentage of STEC isolation in wild and companion birds. In total, 2.28% (5/219) *E. coli* strains were detected, of which 4 (80%) and one (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, and all of them (100%) were identified as STEC. Both studies demonstrated a high frequency of STEC among *eae* positive strains with an increase in their frequency (1.8% (4/219) to 4.5% (9/200)), about 2.5 times more than the previous study, in which the *stx*2 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 HUS. So, further study is needed to investigate the severity of their pathogenicity as a zoonotic pathogen. According to a study conducted by Zahraei *et. al*. (2007) among 12 APEC isolates that belonged to the most common serotypes in Iran, the *stx*2 gene was detected in 75% (9/12) of isolates, while only 8.3% (1/12) possessed both *stx*1 and *stx*2. Furthermore, 16.66% of isolates possessed the *eae* gene. This study's results demonstrated that the *stx*2 virulence factor may be widespread among APEC in Iran, which is in coordination with our study because we detected *stx*2 from all isolated STEC strains. Zarei *et al*. (2019) investigated the prevalence of STEC in 257 raw chicken meat samples collected. In total, 36% (93/257) *E. coli* were found, with STEC, EPEC, and AEEC accounting for

38.7% (36/93), 7.5% (7/93), and 12.9% (12/93), 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 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, 2013), raw milk (Momtaz *et al*., 2012; Mohammadi, 2013.; Brenjchi, 2011), bovine subclinical mastitis milk (Ahmadi *et al*., 2019), minced meat (Kazemi, 2012), carcasses of sheep (Jafareyan-Sedigh, 2011), hamburger (Jamshidi, 2012; Kargar, 2013), water and vegetables (Shah Illi, 2010), lettuce (Mazaheri S, 2014), beef meat (Sami, 2007), etc (Hooman, *et al.,* 2020).

Chiacchio *et al*. (2016) collected 171 fecal samples from Psittaciformes (67 cockatiels, 59 budgerigars, and 45 Agapornis). They identified 42 (24.5%) *E. coli* strains, among which 19.4% (8/42) were determined to be positive for the *eae* and *stx*2 genes. Isolated STEC strains were detected with a percentage of 8.47% (5/59) in budgerigars, 4.47% (3/67) in cockatiels, and 0% (0/45) in Agapornis. The majority of STEC isolates in their study belong to budgerigars, while in our study, 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 in 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). 77.7% (401/ 516) *E. coli* strains were detected from the collected fecal samples. Multiplex PCR for detection of *eae*, *bfp*A, *stx*1, and *stx*2 genes was done on *E. coli* isolates. 23 (5.7%), 16

(3.9%), and 3 (0.7%) out of 401 *E. coli* strains were positive for the presence of *eae*, *bfp*A, and *stx*2 genes, respectively. None of the strains were positive for the *stx*1 gene. Based on their results, the prevalence of STEC, tEPEC, and aEPEC is significant and should be considered for their zoonotic potential. The orders in which AEEC was discovered were Psittaciformes (13/99), Strigiformes (1/48), and Columbiformes (9/72), while our study revealed that only 4 out of 150 (2.6%) of 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 were inquiring about the occurrence of AEEC in pet birds, but it should be investigated 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% (7/9) of the isolated strains in this study, because these strains demonstrated resistance to three or more classes of examined antibiotics (Chiacchio *et al*., 2016).

A high prevalence of resistance was found to amoxiclav (7/9), chloramphenicol (6/9), and streptomycin (6/9), although all of the recovered isolates (9/9) 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 human (children or adults) fecal samples has been investigated in several studies. According to a review article, 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, 2009). This evidence reveals a significant frequency of STEC in gastroenteritis patients (Hooman *et al.,* 2020). 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, 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, while three (Cockatiel, Mynah, and White-eared bulbul) were classed as phylogroup D. Two strains, one from a duck and the other from a cockatiel, were not typable. In a study done by 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 for 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 indicates the importance of paying more attention to this agent as a risk to public health, particularly children and those people who suffer from immunosuppression diseases. Furthermore, the high rate of 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*) establish STEC as a considerable public health threat.

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) جدا شده از پرندگان زینتی در AEECآنالیز فیلوژنتیکی سویه های اشرشیاکلی اتصال و آسیب (

ایران

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زمینه مطالعه: انتروپاتوژنیک اشرشیاکلی (EPEC) و اشرشیاکلی های تولیدکننده شیگاتوکسین (STEC) به دلیل دارا بودن ژن *eae* جز اشرشیاکلی های اتصال و آسیب دسته بندی می شوند. AEEC یکی از عوامل مهم اسهال در انسان ها هستند که می توانند پرندگان را هم درگیر کنند و باید به عنوان یک عامل بیماری زای قابل انتقال بین انسان و حیوانات مورد توجه قرار گیرند.

هدف: هدف از مطالعه ما بررسی حضور سویه های AEECدر پرندگان زینتی، بررسی میزان مقاومت آنتی بیوتیکی آن‌ها و تعیین گروه های فیلوژنتیکی سویه های جدا شده بود.

روش کار: در مجموع 200 نمونه مدفوعی از پرندگان زینتی ارجاعی به بخش پرندگان زینتی بیمارستان دامپزشکی دانشگاه تهران جمع آوری شد. نمونه های مشکوک به اشرشیاکلی از نظر حضور ژن های *usp*A، *eae*، *bfp*A، *stx*1، *stx*2 مورد بررسی قرار گرفتند. در مرحله بعد گروه های فیلوژنتیکی سویه های AEEC جدا شده تعیین گردیدند. در مرحله آخر مقاومت آنتی بیوتیکی سویه های مذکور با کمک دو روش آگار دیسک دیفیوژن و حداقل غلظت مهاری مورد بررسی قرار گرفتند.

نتایج: به طورکلی، 26 سویه اشرشیاکلی (13٪) از نمونه های جمع آوری جداسازی شد. از این میان، 9 نمونه دارای ژن *eae* بودند، و هیچ یک از آن ها ژن *bfp*A را نداشتند. 4 نمونه تنها دارای ژن حدت *stx*2 و 5 نمونه دارای هر دو ژن حدت *stx*1 و *stx*2 بودند. گروه فیلوژنتیکی 7 سویه از 9 سویه AEEC قابل تشخیص بود که شامل 4 مورد گروه فیلوژنتیکی B2 و 3 مورد گروه فیلوژنتیکی D بودند. در این مطالعه مقاومت آنتی بیوتکی چندگانه (MDR) در 77/7 نمونه ها مشاهده شد.

نتیجه گیری نهایی: شناسایی سویه‌های AEEC در پرندگان زینتی (به عنوان یکی از رایج ترین حیوانات خانگی در ایران که دارای ارتباط نزدیک با صاحب خود به ویژه کودکان است) دارای گروه فیلوژنتیکی مشترک با سویه‌های جدا شده از انسان، همچنین مقاومت آن‌ها به طیف وسیعی از آنتی بیوتیک‌های مورد استفاده در طب انسانی، بیانگر اهمیت مطالعه روی AEEC ها به عنوان تهدیدی جدی برای سلامت عمومی می باشد.

واژه‌های کلیدی: اشرشیاکلی، اشرشیاکلی اتصال و آسیب، اشرشیاکلی تولیدکننده شیگاتوکسین، آنالیز فیلوژنتیکی، شیگاتوکسین

Table 1. The list of investigated avian species along with detected virulence genes (*eae*, *stx*1, and *stx*2), and phylogroups in them

No.	Order	Bird species	No. of samples	*E. coli* +	*eae* +	*stx*1 +	*stx*2 +	*bfp*A +	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	9 (4.5%)	5 (2.5%)	9 (4.5%)	0 (0%)	-

	(13%)

435

440

445

450 **Table 2.** The list of primers and their sequences was 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	*arpA*	F. AACGCTATTCGCCAGCTTGC	400	Clermont *et al.* (2013)
	ArpA1.r		R. TCTCCCCATACCGTACGCTA		
	chuA.1b	*ChuA*	F. ATGGTACCGGACGAACCAAC	288	
	chuA.2		R. TGCCGCCAGTACCAAAGACA		
	yjaA.1b	*yjaA*	F. CAAACGTGAAGTGTCAGGAG	211	
	yjaA.2b		R. AATGCGTTCCTCAACCTGTG		
	TspE4C2.1b	*TspE4C2*	F. CACTATTCGTAAGGTCATCC	152	

	TspE4C2.2b		R. AGTTTATCGCTGCGGGTCGC		
Group E	ArpAgpE.f	*arpA*	F. GATTCCATCTTGTCAAAATATGCC	301	
	ArpAgpE.r		R. GAAAAGAAAAAGAATTCCCAAGAG		
Group C	trpAgpC.1	*trpA*	F. AGTTTTATGCCCAGTGCGAG	219	
	trpAgpC.2		R. TCTGCGCCGGTCACGCCC		
Internal control	trpBA.f	*trpA*	F. CGGCGATAAAGACATCTTCAC	489	
	trpBA.r		R. GCAACGCGGCCTGGCGGAAG		
Group G	ybgD.f	*ybgD*	F. GTTGACTAARCGYAGGTCGA	567	Clermont *et al.* (2019)
	ybgD.r		R. KATGYDGCYGATKAAGGATC		

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Table 3. The results of antibiotic resistance profile of isolated strains by using disk diffusion (DD) and minimum inhibitory concentration (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	-		