ORIGINAL ARTICLE

Veterinary Research Forum. 2018; 9 (3) 211 - 216
doi: 10.30466/vrf.2018.30827
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

ECOR phylotyping and determination of virulence genes in *Escherichia coli* isolates from pathological conditions of broiler chickens in poultry slaughter-houses of southeast of Iran

Akbar Asadi[1], Taghi Zahraei Salehi[2], Mahmoud Jamshidian[3], Reza Ghanbarpour[4]*

[1] Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran ;[2] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [3] Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran; [4] Molecular Microbiology Research Group, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Article Info

Article history:

Received: 17 June 2017
Accepted: 09 January 2018
Available online: 15 September 2018

Key words:

Cellulitis
Colibacillosis
Escherichia coli
Phylogenetic group
Virulence genes

Abstract

Avian pathogenic *Escherichia coli* (APEC) are responsible for wide ranges of extra-intestinal diseases in poultry including colibacillosis, cellulitis, coligranuloma and yolk sac infection. Numbers of virulence are considered important in the pathogenicity of these diseases. The aims of the present study were phylogenetic typing and virulence genes detection in *Escherichia coli* isolates from colibacillosis and cellulitis of broiler chickens in poultry slaughterhouses of Shahrbabak region, Kerman, Iran. A total number of eighty three *E. coli* isolates were taken from broiler chickens with colibacillosis and thirty four isolates were taken from carcasses with cellulitis in the industrial slaughterhouses. Biochemically confirmed *E. coli* isolates were subjected to polymerase chain reaction assay to determine phylogenetic groups and presence of *pap C*, *sfa/focDE*, *iucD*, *afaIB-C*, *hlyA*, *fimH* and *crl* virulence genes. Colibacillosis isolates were belonged to A (54.21%), B1 (7.22%), B2 (6.03%) and D (32.53%) phylogroups. Whereas, the isolates from cellulitis cases were belonged to three main phylogroups; A (55.88%), B1 (5.88%) and D (38.24%). Statistical analysis showed a specific association between the presence of *crl* virulence gene and phylogroups of A and D in colibacillosis isolates. The results showed that the isolates from both diseases in broiler chickens could be assigned to various phylogenetic groups (mainly A). Also, the virulence genes profile of cellulitis *E. coli* is completely different from that of colibacillosis in this region.

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تعیین زمینه فیلوژنتیکی و بررسی حضور ژن های حدت در *اشریشیاکلی* جدا شده از شرایط پاتولوژیک طیور گوشتی درکشتارگاه های طیور جنوب شرق ایران

چکیده

اشریشیاکلی بیماری زای طیور (APEC) مسئول طیف وسیعی از بیماری های خارج روده ای در طیور شامل کلی باسیلوز، سلولیت، کلی گرانولوما و عفونت کیسه زرده می باشد. تعدادی از عوامل حدت در بیماری زایی این بیماری ها مهم در نظر گرفته می شوند. اهداف مطالعه حاضر، تعیین زمینه فیلوژنتیکی و بررسی حضور ژن های حدت در *اشریشیاکلی* جدا شده از کلی باسیلوز و سلولیت جوجه های گوشتی در کشتارگاه های طیور منطقه شهربابک استان کرمان ایران بودند. تعداد ۸۳ جدایه *اشریشیاکلی* از جوجه های گوشتی دارای کلی باسیلوز و ۳۴ جدایه از لاشه های واجد سلولیت در کشتارگاه های صنعتی به دست آمد. جدایه های تأیید شده با روش بیوشیمیایی به منظور تعیین گروه های فیلوژنتیکی و بررسی حضور ژن های حدت *pap C*، *sfa/focDE*، *iucD*، *afaIB-C*، *hlyA*، *fimH* و *crl* به روش واکنش زنجیره ای پلیمراز مورد بررسی قرار گرفتند. جدایه های *مربوط به موارد کلی باسیلوز* متعلق به گروه های فیلوژنتیکی A (۵۴/۲۱ درصد)، B1 (۷/۲۲ درصد)، B2 (۶/۰۳ درصد) و D (۳۲/۵۳ درصد) بودند. در حالی که جدایه های حاصل از موارد سلولیت به سه گروه اصلی فیلوژنتیکی A (۵۵/۸۸ درصد)، B1 (۵/۸۸ درصد) و D (۳۸/۲۴ درصد) تعلق داشتند. ارزیابی آماری ارتباط ویژه ای بین حضور ژن حدت *crl* و گروه های فیلوژنتیکی A و D را در جدایه های حاصل از کلی باسیلوز نشان داد. نتایج نشان داد که جدایه های هر دو بیماری در جوجه های گوشتی می توانند در گروه های فیلوژنتیکی مختلف، عمدتاً A، تقسیم بندی شوند. همچنین، پروفایل ژن های حدت در *اشریشیاکلی* جدا شده از موارد سلولیت با پروفایل موارد کلی باسیلوز در این منطقه کاملاً متفاوت بود.

واژه های کلیدی: *اشریشیاکلی*، ژن های حدت، سلولیت، کلی باسیلوز، گروه فیلوژنتیکی

***Correspondence:**

Reza Ghanbarpour. DVM, PhD
Molecular Microbiology Research Group, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
E-mail: ghanbar@uk.ac.ir

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Introduction

Assessment of the evolutionary origins via phylogenetic analysis has been helpful for determination of the pathogenic and/or non-pathogenic nature of *E.* coli isolates. The *E. coli* can be categorized into four recognized phylogenetic groups; A, B1, B2 and D. This classification has been performed using a triplex polymerase chain reaction (PCR) based method defined previously based on detection of three genetic sequences called *chuA*, *yjaA* and TSPE4.C2.[1]

Avian pathogenic *Escherichia coli* (APEC) are responsible for wide ranges of extra-intestinal diseases in poultry including colibacillosis, cellulitis, coligranuloma, yolk sac infection, airsacculitis and omphalitis.[2] Cellulitis is characterized by a necrotic dermatitis of thighs and abdomen.[3] Avian cellulitis and colibacillosis are considered as the most important economic threats to poultry industry.[4] Reportedly, *E. coli* isolates from avian cellulitis harbor similar virulence factors with other colibacillosis lesions.[5]

Numbers of virulence factors including adhesins, toxins, anti-host defense factors, iron acquisition systems, autotransporters and the IbeA protein are involved in these pathological conditions.[6]

Existence of adhesions factors such as S and P fimbriae is considered to be an essential factor in pathogenesis of these isolates due to their abilities to adhere the poultry epithelial cells. The P fimbriae are important factors for the beginning and expansion of human urinary tract infections; however their roles in the pathogenesis of avian isolates have not been completely clarified. Also, curli fimbriae (encoded by *crl* and *csgA* genes) facilitate *E. coli* isolates adherence to fibronectin and laminin, but their roles in the pathogenesis of APEC are not certainly understood.[7] Aerobactin production which facilitates iron scavenging by *E. coli* has been related to pathogenicity of avian *E. coli.* The *iucD* gene encoding aerobactin secretion is highly prevalent virulence gene in APEC isolates.[8]

Phylogenetic typing of broiler chickens with colibacillosis in Ahvaz (southwest of Iran) has showed that these isolates are mainly belonged to A phylotype.[9] Phylotyping and ColV plasmid-associated virulence genotyping of *E. coli* isolated from broiler chickens with colibacillosis in Fars province, Iran, were also investigated.[10] However, the link between avian *E. coli* pathogenicity and different phylotypes and genotypes might provide useful characteristics for the better Iranian APEC identification.

The aim of the present study was phylogenetic typing and virulence genes detection in *Escherichia coli* isolates from pathological conditions of broiler chickens in poultry slaughterhouses of Shahrbabak region, Kerman, Iran.

Materials and Methods

Sample collection and *E. coli* isolation. A total number of 83 *E. coli* isolates were taken from broiler chickens with colibacillosis between November 2014 to April 2015 and thirty four *E. coli* isolates were taken from broiler carcasses with cellulitis between November 2014 to September 2015. The samples were taken from an industrial poultry slaughterhouse in Kerman province (southeast of Iran). For the initial enrichment, the samples were inoculated into 3 mL buffered peptone water (Merck, Darmstadt, Germany) and incubated at 37 ˚C for 5 to 6 hr. Subsequently, the enriched samples were streaked on MacConkey agar (Merck) and incubated at 37 ˚C overnight. Biochemical confirmations were performed on suspected colonies using indole, methyl red, Voges-Proskauer and citrate tests[11] and finally confirmed *E. coli* isolates were subjected to PCR assays.

PCR assay for virulence genes detection. The DNA extractions of overnight cultures of *E. coli* isolates and reference isolates were prepared by lysis method.[12] The isolates were examined through PCR for the presence of curli fimbriae (*crl*), P fimbriae (*pap C*), S fimbriae (*sfa/focDE*), aerobactin (*iucD*), afimbrial adhesin Afa (*afaIB-C*), haemolysine (*hlyA*) and F1 fimbriae (*fimH*) encoding genes.[6,13,14] Reference isolates used as positive controls for specific virulence genes were included 1404 (f17A), 239KH89 (*iucD* and *afaE-VIII*), A30 (*afaIB-C* and *hlyA*) and J96 (*sfa/focDE, papC, fimH* and *crl*). Laboratory non-pathogenic *E. coli* isolate MG1655 was used as a negative control.

Phylotyping analysis. Phylogenetic groups were identified by multiplex PCR based on the presence or absence of *chuA*, *yjaA* and TSPE4.C2 sequences in each *E. coli* isolates.[1] The *E. coli* isolates were segregated in four distinct phylogenetic groups; A, B1, B2 and D. Four *E. coli* isolates from the *Escherichia coli* reference (ECOR) collection were used as controls for phylogenetic determination; ECOR58 (B1 group), ECOR62 (B2 group), ECOR50 (D group) and *E. coli* isolate MG1655 as a positive control for phylogenetic ECOR group A. The primers used for virulence genes amplification as well as phylogenetic groups are shown in Table 1. All the reference isolates were provided from the bacterial collection of Microbiology Department of École Nationale Vétérinaire de Toulouse, Toulouse, France.

Statistical analysis. The data were analyzed using SPSS (version 17.0; SPSS Inc., Chicago, USA) and *p* values were calculated using Chi-square and Fisher's exact tests to find any significant relationship. The *p* values less than 0.05 were considered statistically significant.

Results

In total, phylogenetic analysis of 117 *E. coli* isolates from pathological conditions of broiler chickens showed that these isolates were divided into four phylogenetic groups as follows: 64 isolates (54.70%) into A, 40 isolates (34.18%) into D, eight isolates (6.83%) into B1 and five

isolates (4.29%) into B2 phylogroups. Virulence genotyping of *E. coli* isolates showed that none of the isolates harbored the *afaI B-C, afa E-VIII, hly* and *f17A* genes. The *Crl, fimH, papC* and *sfa/foc* were the most prevalent patterns of the isolates (40/117, 34.18%) and most of these isolates (27/40, 67.50%) were belonged to A phylogenetic group. Seventeen isolates (17/117, 14.52%) didn't have any virulence genes. Statistical analysis showed a specific association between the presence of *crl* gene and phylogroups of A and D ($p < 0.05$). In addition, a specific association between *crl* gene and *fimH sfa/foc* was found ($p < 0.05$). Details of detected patterns of virulence genes in relation to the phylogenetic subgroups/groups of *E. coli* isolates from colibacillosis and cellulitis cases of broiler chickens are shown in Table 2.

Table 1. Primers used to detection of virulence genes and phylogenetic groups.

Target	Sequence (5'-3')	Size (bp)	Ref.
iucD	TACCGGATTGTCATATGCAGACCG AATATCTTCCTCCAGTCCGGAGAAG	602	13
hly	AACAAGGATAAGCACTGTTCTGGC ACCATATAAGCGGTCATTCCCGTCA	1,177	13
Sfa/focDE	CGGAGGAGTAATTACAAACCTGGCA CGGAGGAGTAATTACAAACCTGGCA	410	13
afaI B-C	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	13
pap C	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	13
afa E-VIII	CTAACTTGCCATGCTGTGACAGTA TTATCCCCTGCGTAGTTGTGAATC	310	14
crl	TTTCGATTGTCTGGCTGTATG CTTCAGATTCAGCGTCGTC	250	18
Fim H	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	6
ChuA	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	1
Yja A	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	1
TspE4C2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	1

Phylotype analysis indicated that eighty three *E. coli* isolates from heart blood samples of broiler chickens with colibacillosis (septicemic isolates) were belonged to four main groups; A (54.21%), B1 (7.22%), B2 (6.03%) and D (32.53%). Phylogenetic subgroups classification revealed that the predominant phylogroup in colibacillosis *E. coli* was A0 (38.50%), whereas 51 isolates (61.44 %) fell into six phylogenetic subgroups as follows: 13 isolates (15.60%) into A1, six isolates (7.20%) into B1, four isolates (4.81%) into B2-2, one isolates (1.20%) into B2-3, 26 isolates (31.32%) into D1 and one isolate (1.20%) into D2.

Multiplex and simplex PCR assays showed that out of 83 *E. coli* isolates from colibacillosis cases, 62 isolates (74.69%), 57 isolates (68.67%) and 53 isolates (63.85%) were positive for *crl*, *fimH* and *sfa/foc* genes, respectively. These three genes were the three most prevalent detected virulence genes. Among 62 *crl*-positive isolates, 40 (64.51%) isolates were in combination with F1, S and P fimbriae encoding genes (Fig. 1). The *fimH* gene as the second most prevalent adhesion gene was found in 57 (68.67%) isolates. All of the *fimH*-positive isolates had one of the other examined genes. The *iucD* gene was found in 2.56% of isolates (Fig. 2). Eight different combination patterns of the virulence genes were detected among colibacillosis isolates (Table 2).

The *E. coli* isolates from carcasses with cellulitis were belonged to three main phylogenetic groups; A (55.88%), B1 (5.88%) and D (38.24%). The predominant phylogroup in *E. coli* isolates was A0 (50.00%) and the rest of isolates (17/34) were divided into A1 (5.88%), B1 (5.88%), D1 (11.76%) and D2 (26.48%).

Table 2. Patterns of detected virulence genes in relation to the phylogenetic subgroups/groups of *E. coli* isolates from colibacillosis and cellulitis cases of broiler chickens.

Phylogenetic group		A		B1	B2		D		Total No. (%)
Phylogenetic subgroup		A0	A1	B1	B2-2	B2-3	D1	D2	
Patterns of detected genes	**APEC**								
crl fimH papC sfa/foc	Colibacillosis	23	4	3	-	-	10	-	40 (34.18)
crl fimH sfa/foc	Colibacillosis	3	3	-	-	-	-	1	7 (5.98)
crl fimH iucD	Colibacillosis	1	2	-	-	-	-	-	3 (2.56)
crl- fimH	Colibacillosis	3	-	1	1	-	-	-	5 (4.27)
crl- papC	Colibacillosis	-	3	1	2	-	1	-	7 (5.98)
fimH-papC	Colibacillosis	-	-	-	-	-	1	-	1 (0.85)
fimH-sfa/foc	Colibacillosis	1	-	-	-	-	-	-	1 (0.85)
papC-sfa/foc	Colibacillosis	1	1	1	1	1	-	-	5 (4.20)
fimH	Cellulitis	16	-	2	-	-	-	3	21 (17.90)
crl	Cellulitis	-	2	-	-	-	4	3	9 (7.69)
papC	Cellulitis	1	-	-	-	-	-	-	1 (0.85)
Non detected		-	-	-	-	-	14	3	17(14.52)
Total phylosubgroup		49	15	8	4	1	30	10	117
Total phylogroup		64	-	8	5	-	40	-	117

APEC: Avian pathogenic *Escherichia coli*.

The prevalence of *fimH* gene among thirty four *E. coli* isolates from carcasses with cellulitis was (61.76%), (Fig. 3). The frequency of *crl* and *papC* genes was 26.74% and 2.95%, respectively. These isolates were negative for the other examined genes (Table 2).

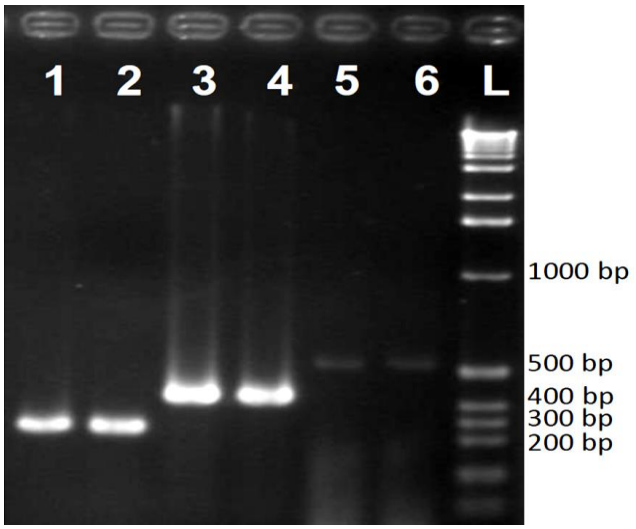

Fig. 1. PCR results for *crl*, *fimH* and *sfa/foc* genes detected from colibacillosis isolates. Lane L: Ladder 1 kb; Lane 1: Positive control for *crl* gene (*E. coli* J96); Lane 2: Positive isolate for *crl* gene; Lane 3: Positive control for *sfa/foc* DE gene (*E. coli* J96); Lane 4: Positive isolate for *sfa/foc* DE gene; Lane 5: Positive control for *fimH* gene (*E. coli* J96); Lane 6: Positive isolate for *fimH* gene.

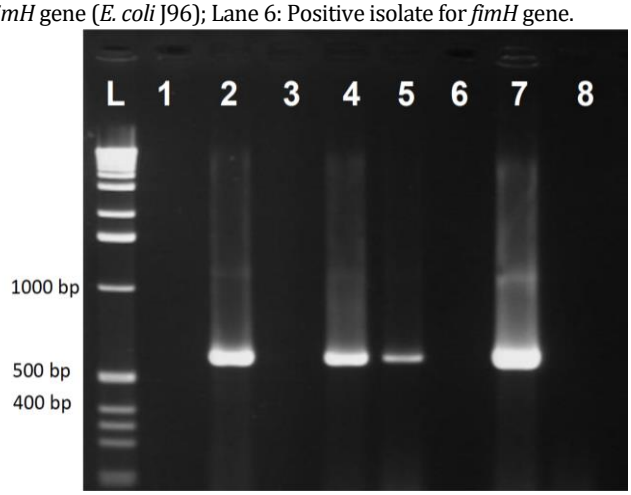

Fig. 2. The PCR result for *iucD* gene detected from colibacillosis isolates. Lane L: Ladder 1 kb; Lane 1: Negative control (*E. coli* MG1655); Lane 2: Positive control for *iucD* gene (*E. coli* 239KH89); Lane 4, 5and 7: Positive isolates for *iucD* gene.

Discussion

Genotyping of APEC and evolutionary findings of the adhesive capacity of *E. coli* isolates isolated from avian colibacillosis and cellulitis showed that these isolates can be categorized into different phylogenetic groups.[5,10] In the present study, phylogenetic analysis of 117 *E. coli* from colibacillosis and cellulitis showed that

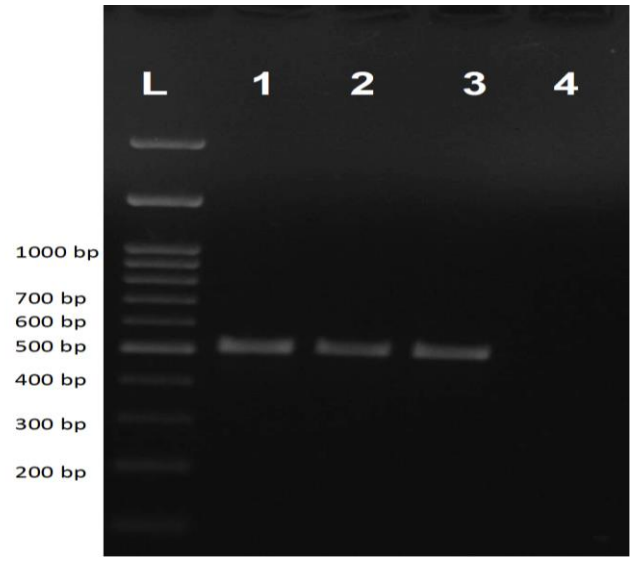

Fig. 3. The PCR result for *fimH* gene detected from cellulitis isolates. Lane L: Ladder 1 kb; Lane 1: Positive control for *fimH* gene (*E. coli* J96); Lane 2 and 3; Positive isolates for *fimH* gene; Lane 4: Negative control (*E. coli* MG1655).

most of the isolates harboring virulence genes were belonged to A phylogroup. Another study on phylogenetic groups in *E. coli* isolates isolated from septicemic broiler and layer cases has shown that the APEC isolates were belonged to A (71.00%), B1 (4.10%), B2 (7.90%) and D (18.70%) phylo-groups.[15] The data about phylogenetic groups of APEC especially isolates from colibacillosis and cellulitis are limited. It has been reported that the isolates from these pathological conditions are mostly belonged to A phylogroup and in the lesser amount to D group which is in accordance with our study.[16]

Although previous reports have found that APEC isolates can cause serious infections (both localized and systemic infections) in birds, the pathophysiology of these infections has not been elucidated.[6]

It seems that important stages in the pathogenicity of APEC isolates are fimbrial and putative virulence factors and the expression of these factors can be associated with resistance to host immune response. Previous studies have found that virulence profile and molecular characteristics of *E. coli* isolates from avian colibacillosis and avian cellulitis are similar,[17] but in the present study, the detected gene patterns in colibacillosis cases had higher diversity than cellulitis *E. coli.*

Several studies have showed sequence homology between specific DNA regions of APEC and human extra-intestinal pathogenic *E. coli* (ExPEC).[18] Moreover, the presence of similar virulence genes in APEC and ExPEC isolates proposed that APEC isolates can act as zoonotic pathogens and reservoirs of virulence causing human infections.[18] It has been reported that the isolate can be considered as ExPEC if contains two or more of the following virulence genes; *pap* (P fimbriae), *sfa/foc* (S/F1C

fimbriae), *afa/dra* (binding adhesins), *iutA* (aerobactin receptor) and *kpsM II* (group 2 capsule synthesis).[3]

The prevalence of type 1 fimbrial adhesion gene (*fimH*) in APEC isolates was 66.66% in this study. The FimH virulence factor is seemed to be an essential unit for protecting the APEC isolates against host immune system,[17] but the exact role of Fim H in the pathogenesity of APEC isolates remains debatable with incompatible results.[19] Other studies have shown higher prevalence of the *fimH* virulence gene.[20]

According to the results of the present study, one hundred isolates (100/117, 85.47%) harbored at least one of the examined genes, whereas; the *crl* gene was the second most prevalent gene (71/117, 60.68%) among APEC isolates. It has been shown that curli-negative mutant isolates have less adherence colonization, invasion and persistence to chicken tissues recommending curli as a virulence factor.[21] It has been reported that the prevalence of *crl* gene among APEC isolates was 92.70% in Germany.[16] Therefore, it can be supposed that most of APEC isolates are curliated.[20]

In the present study, the prevalence of the *pap* gene (53/83) in colibacillosis *E. coli* was remarkably higher than that of cellulitis *E. coli* (1/34). The reason for higher association between *pap* gene and colibacillosis *E. coli* has not been yet notified. Accordingly, the *pap* operon genes in less than 20.00% of the APEC have been identified in broiler chickens previously.[22]

None of the studied isolates were positive for *afaI B-C, afa E-VIII* and *f17A* genes. Previous studies have showed that the *Afa* adhesions exist on less than 10.00% of APEC isolates suggesting that *E. coli* isolates expressing *afa-8* gene are able to reproduce clinical symptoms and lesions of cellulitis and collibacillosis.[16]

In this study, fifty three of the isolates were positive for *sfa* genes among colibacillosis isolates and none of the cellulitis-related isolates were positive for this gene. While, the *sfa* gene was identified in the range of 0.00%, 1.80% and 8.80% of isolates from APEC by PCR method.[16,22,23] Previous study has showed that the S fimbria can be found rarely in APECs and its role in the pathogenicity of them is not completely clarified.[23]

In the present study, 3 (3.60%) of *E. coli* isolates from colibacillosis cases were considered as *iucD*-positive and none of the isolates from cellulitis cases were positive for *iucD* gene. These findings are in contrast with the frequencies of aerobactin genes in previous studies ranged from 44.00% to 83.00% in the isolates from coliform cellulitis and colibacillosis.[24]

In conclusion, *E. coli* isolates from colibacillosis and cellulitis cases were disseminated in various phylogenetic groups (mainly A). These isolates harbored various fimbrial genes in a relatively high prevalence. On the other hand, the virulence profile of cellulitis *E. coli* was different from that of colibacillosis cases. However, these aforementioned pathological conditions may be associated with other virulence factors that have not been investigated. More researches are needed to determine the phylogenetic analysis of APEC isolates from other pathological conditions to find the exact relation between the virulence genes and ECOR background of these isolates.

Acknowledgments

This work was supported by a grant (No.: 17216-2016/05/18) from Azad University, Shahrbabak Branch, Kerman, Iran.

Conflict of interest

The authors declare that thre is no conflict of interest.

References

1. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2010; 66: 4555-4558.
2. Saif YM, Fadly AM, Glisson JR, et al. Diseases of poultry. 12th ed. London, UK: Blackwell 2008;121-123.
3. Johnson JR, Murray AC, Gajewski A, et al. Isolation and molecular characterization of nalidixic acid-resistant extra-intestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother 2003; 47: 2161-2168.
4. Roy P, Purushothaman V, Koteeswaran A, et al. Isolation, characterization, and antimicrobial drug resistance pattern of *Escherichia coli* isolated from Japanese quail and their environment. J Appl Poultry Res 2006; 15(3): 442-446.
5. de Brito BG, Gaziri LC, Vidotto MC. Virulence factors and clonal relationships among *Escherichia coli* isolates isolated from broiler chickens with cellulitis. Infect Immun 2003; 71:4175-4177.
6. Ghanbarpour R, Salehi M, Oswald E. Virulence genotyping of *Escherichia coli* isolates from avian cellulitis in relation to phylogeny. Comp Clin Pathol 2010; 19:147-153.
7. de Campos TA, Stehling EG, Ferreira A, et al. Adhesion properties, fimbrial expression and PCR detection of adhesin-related genes of avian *Escherichia coli* isolates. Vet Microbiol 2005; 106(3): 275-285.
8. Kawano M, Yaguchi K, Osawa R. Genotypic analyses of Escherichia coli isolated from chickens with colibacillosis and apparently healthy chickens in Japan. Microbiol Immunol 2006; 50: 961-966.
9. Jafari RM, Motamedi H, Maleki E, et al. Phylogenetic typing and detection of extended-spectrum β-lactamases in Escherichia coli isolates from broiler chickens in Ahvaz, Iran. Vet Res Forum 2016; 7(3): 227-233.

10. Mohsenifard E, Asasi K, Sharifiyazdi H, et al. Phylotyping and ColV plasmid-associated virulence genotyping of *E. coli* isolated from broiler chickens with colibacillosis in Iran. Com Clin Patho 2016; 25: 1035-1042.
11. Ghanbarpour R, Askari N, Ghorbanpour M, et al. Genotypic analysis of virulence genes and antimicrobial profile of diarrheagenic *Escherichia coli* isolated from diseased lambs in Iran. Trop Anim Health Prod 2017; 49(3):591-597.
12. Alizadeh H, Ghanbarpour R, Jajarmi M, et al. Phylogenetic typing and molecular detection of virulence factors of avian pathogenic *Escherichia coli* isolated from colibacillosis cases in Japanese quail. Vet Res Forum 2017; 8(1):55-58.
13. Yamamoto S, Terai A, Yuri K, et al. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. FEMS Immunol Med Microbiol 1995; 12(2): 85-90.
14. Van Bost S, Jacquemin E, Oswald E, et al. Multiplex PCRs for identification of necrotoxigenic *Escherichia coli*. J Clin Microbiol 2003; 41:4480-4482.
15. Dissanayake DR, Wijeuardana TG, Gunawardena G, et al. Distribution of lipopolysaccharide core type among avian pathogenic *E. coli* in relation to the major phylogenetic groups. Vet Microbiol 2008; 132(3-4):355-363.
16. Ewers C, Li G, Wilking H, et al. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int J Med Microbiol 2007; 297: 163-176.
17. Mellata M. Human and avian extra-intestinal pathogenic *Escherichia coli*: Infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis 2013; 10(11): 916-932.
18. Mora A, Viso S, López C, et al. Poultry as reservoir for extra-intestinal pathogenic *Escherichia coli* O45:K1:H7-B2-ST95 in humans. Vet Microbiol 2013; 167(3-4): 506-512.
19. Li G, Laturnus C, Ewers C, et al. Identification of genes required for avian *Escherichia coli* septicemia by signature-tagged mutagenesis. Infect Immun 2005; 73: 2818-2827.
20. La Ragione RM, Woodward MJ. Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia. Res Vet Sci 2002; 73: 27-35.
21. Mokady D, Gophna U, Ron EZ. Virulence factors of septicemic *Escherichia coli* isolates. Int J Med Microbiol 2005; 295(6-7); 455-462.
22. Delicato ER, de Brito BG, Gaziri LC, et al. Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. Vet Microbiol 2003; 94: 97-103.
23. Dozois CM, Fairbrother JM, Harel J, et al. pap- and pil-related DNA sequences and other virulence determinants associated with *Escherichia coli* isolated from septicemic chickens and turkeys. Infect Immun 1992; 60: 2648-2656.
24. Jeffrey JS, Nolan LK, Tonooka KH, et al. Virulence factors of *Escherichia coli* from cellulitis or colisepticemia lesions in chickens. Avian Dis 2002; 46(1): 48-52.