ORIGINAL ARTICLE

Veterinary Research Forum. 2022; 13 (4) 569 - 576
doi: 10.30466/vrf.2021.527563.3160
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Study on antibiotic resistance and phylogenetic comparison of avian-pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC) isolates

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Article Info

Article history:

Received: 09 April 2021
Accepted: 16 June 2021
Available online: 15 December 2022

Keywords:

E. coli pathotypes
ERIC-PCR
Resistance genes

Abstract

Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *E. coli* (UPEC) can cause vast infections in humans and poultry. The present study was conducted to compare the isolates of the APEC and UPEC pathotypes on the basis phenotypic and genotypic features of antibiotic resistance and phylogenetic differences. Total number of 70 identified *E. coli* strains, including 35 APEC and 35 UPEC isolates, were isolated from avian colibacillosis and human urinary tract infection (UTI), and were subjected to the antimicrobial susceptibility testing, polymerase chain reaction (PCR) detection of the resistance genes, phylogenetic grouping and DNA fingerprinting with enterobacterial repetitive intergenic consensus PCR (ERIC - PCR) to survey the variability of the isolates. The most resistance rates among all *E. coli* isolates were, respectively, obtained for Ampicillin (84.20%) and sulfamethoxazole-trimethoprim (65.70%). The APEC and UPEC isolates showed the most susceptibility to imipenem and gentamycin, respectively. Among 70 APEC and UPEC isolates 34.20%, 32.80%, 20.00%, and 12.80% belonged to the A, B2, D, and B1 phylogenetic groups, respectively. Analysis of the DNA fingerprinting phylogenetic tree showed 10 specific clusters of APEC and UPEC isolates. According to the results, the most effective antibiotics and the phenotypic and genotypic predominant resistance patterns of the APEC and UPEC isolates were different. Moreover, APECs and UPECs showed various dominant phylogenetic groups. With all descriptions, the APEC isolates still are potential candidates for carrying important resistance genes and can be one of the possible strains related to human infections.

Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains convey resistance and virulence factors which cause the endurance and growth of the bacterium outside of their host intestine. Among various types of ExPEC, uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC) are, respectively, responsible for urinary tract infections (UTIs) in human and avian colibacillosis with vast economic and health adverse effects on society.[1] Recent studies about genomic characteristics of the APEC and UPEC, such as antibiotic resistance genes and virulence factors indicated that there were similarities and proximity between these types of *E. coli*[2] which had potential risk of zoonotic and pandemic outbreaks.[3] Different antibiotics such as β-lactams, aminoglycosides, sulfonamides and fluoroquinolones are the major choice for avian colibacillosis outbreaks in poultry farms all around the world. The incessant usage of these antibiotics in the cycle of poultry breeding has contributed to the emergence of antimicrobial-resistant *E. coli* in human infections.[4-6] UPEC and APEC possibly will encounter comparable situations in different organs and when launching infection in their hosts, they can share a similar content of resistance and virulence genes and pathogenicity properties which are related to their genotypic features. Therefore, the potential of APEC to function as a source of UPEC and other human ExPEC would need to be investigated.[7-9] Some of mobile elements such as plasmid and transposon dependent resistance genes can horizontally transfer in natural microbial communities.[10,11] With these explanations, the

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present study was conducted to assess the potential of APEC to cause human UTI and determination of possible relationships between these *E. coli* types. Therefore, UPECs and APECs were compared for antibiotic susceptibility, their content of resistance genes, DNA fingerprinting and phylo-genetic groups. Then the results were statistically analyzed to distinguish similarities and diversities between the APEC and UPEC isolates considered in this study.

Materials and Methods

Bacterial isolates and DNA extraction. Samples were collected during summer 2020 from UTI cases referred to laboratories and avian colibacillosis suspected cases with clinical signs referred to the veterinary clinics in Amol city, northern Iran. Sampling was done from total number of 127 cases (including 45 avian colibacillosis infections and 82 UTI cases) using sterile cotton swabs (swabs from respiratory organs, liver and heart of suspected poultry and urine culture for UTI samples). Avian colibacillosis cases were sampled from recently dead animals during autopsy. Samples were cultured immediately on MacConkey and Eosin methylene blue agar (HiMedia Corp., Mumbai, India) and incubated for 24.00 hr at 37.00 ˚C. Suspected colonies were identified by gram staining and biochemical tests according to the standard procedures.[12,13] Totally, 70 isolates containing 35 APEC and 35 UPEC were isolated using cultivation and biochemical methods. DNA extraction from *E. coli* isolates was done using gram negative DNA extraction kit (Sinaclon, Tehran, Iran) according to the manufacturer instructions. The extracted DNA and the identified isolates were stored at – 20.00 ˚C for use in other steps of the study. The *E. coli* ATCC 25922 was used as reference strain in antibiotic susceptibility test and other bacteriological examinations.

Antibiotic susceptibility test. Antimicrobial susceptibility test of *E. coli* isolates was done by Kirby-Bauer disc diffusion method according to the standards set by CLSI.[14] The following antibiotic disks (PadtanTeb; Tehran, Iran) were used with their particular concentrations: Tetracycline (TET; 30.00 µg), erythromycin (ERY; 15.00 µg), trimethoprim (TMP; 5.00 µg), gentamicin (GEN; 10.00 µg), ciprofloxacin (CIP; 5.00 µg), cefoxitin (FOX; 30.00 µg), ceftriaxone (CTR; 30.00 µg), cefepime (FEP; 30.00 µg), imipenem (IMP; 10.00 µg), sulfamethoxazole/trimethoprim (SXT; 1.25/23.75 µg), nalidixic acid (NAL; 30.00 µg) and ampicillin (AMP; 10.00 µg). The plates of Muller Hinton agar (HiMedia Corp.) were incubated at 35.00 ± 2.00 ˚C for 18 hr and diameter of growth inhibition zones was measured and compared to the CLSI standard Tables. As a final point, the rate of multidrug-resistant (MDR) was defined as being resistant to more than three antimicrobial classes.

Detection of antibiotic resistance genes. The isolates were examined for the presence of 12 antibiotic resistance genes in order to determine the potential differences in the presence of the genes in APEC and UPEC isolates (*aac* related to gentamycin resistance, $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1A}$, bla_{VEB} and bla_{SHV} related to β-lactamases, *tetA, tetB* and *tetC* related to tetracycline, *sul1* and *sul2* related to sulfonamides, *dfrA1* related to trimethoprim, *ereA* for erythromycin and *qnrA* for quinolones). Polymerase chain reaction (PCR) was performed using specific primers (Table 1) in the final volume of 25.00 µL including 12.50 µL of a PCR master mix (Sinaclon), 1.00 µL (0.50 µM) of both forward and reverse primers and 2.00 µL of DNA samples that reached to 25.00 µL using distilled deionized water. Then the PCR product was evaluated and confirmed using electrophoresis in 1.50% agarose gel with the assistance of a marker of 100 bp (Sinaclon). Different resistance gene patterns were described according to the presence of the genes.

Phylogenetic group determination. Determination of phylogenetic groups of the APEC and UPEC isolates was done by a multiplex PCR reaction based on the presence of three genetic markers (*chuA*, *yjaA* and *TspE4.C2*) previously described by Clermont *et al*.: $ChuA^{-}$, $YjaA^{-/+}$ and $TSPE4.C2^{-}$ were assigned to group A, $ChuA^{-}$, $YjaA^{-/+}$ and $TspE4.C2^{+}$ were assigned to group B1, $chuA^{+}$, $YjaA^{+}$ and $TspE4.C2^{-/+}$ were assigned to group B2 and $ChuA^{+}$, $YjaA^{-}$ and $TspE4.C2^{-/+}$ were assigned to group D.[15] Multiplex-PCR reaction was done using specific oligonucleotides listed in Table 1.

DNA fingerprinting and phylogenetic tree. Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) was done for DNA fingerprinting comparison of the isolates. ERIC-PCR reactions were performed in final volume of 25.00 µM including 1.50 µL of each primer in final concentration of 2.00 pmol μL^{-1}, 12.50 µL of Master Mix (SinaClon) and 8.50 µL of deionized distilled water. Primer ERIC-1 and primer ERIC-2 were used in ERIC reaction as previously described.[16] The images of ERIC reactions were loaded in BioNumerics (version 6; Applied Maths, Kortrijk, Belgium) for analysis. Genetic similarity was calculated using the Pearson correlation in which 2.00% of the optimization tolerance and 4.00% of the position tolerance shift were set. The dendrogram of the isolates was also created by the Dice correlation coefficient and the un-weighted pair group method with arithmetic averages (UPGMA). A cut-off of 80.00% was used to determine final groupings.

Statistical analysis. We tried to find correlations among different variables, therefore, the results of the study were analyzed using SPSS Software (version 22.0; IBM Corp., Armonk, USA). Statistical analyses were carried out by applying the Mann–Whitney, Chi-square and Kolmogorov - Smirnov tests with a statistically significant p - value < 0.05.

Table 1. Nucleotide sequences used as primers in PCR for identification of resistance genes and phylogenetic grouping among UPEC and APEC isolates.

Target gene	Sequence (5' to 3')	Annealing temperature (˚C)	PCR product size (bp)	Reference
aac(3)	F: CTTCAGGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	55.00	286	33
bla$_{CTX-M-15}$	F: CATGTGCAGYACCAGTAA R: CCGCRATATCRTTGGTGGTG	42.00	542	34
bla$_{TEM-1A}$	F: ATGAGTATTCAACATTTCCG R: CCAATGCTTAATCAGTGAGG	46.00	850	35
bla$_{VEB-19}$	F: CGACTTCCATTTCCCGATGC R: GGACTCTGCAACAAATACGC	51.00	643	36
bla $_{SHV}$	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	52.00	768	36
tetA	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	50.00	210	37
tetB	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	50.00	659	38
tetC	F: CCTCTTGCGGGATATCGTCC R: GGTTGAAGGCTCTCAAGGGC	55.00	505	39
sul1	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	47.00	822	40
sul2	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	51.00	720	40
ereA	F: GCCGGTGCTCATGAACTTGAG R: CGACTCTATTCGATCAGAGGC	52.00	419	33
dfrA1	F: GGAGTGCCAAAGGTGAACAGC R: GAGGCGAAGTCTTGGGTAAAAAC	45.00	367	41
qnrA	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	50.00	516	42
chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	52.00	279	15
YjaA	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	52.00	211	15
TspE4C2	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	50.00	152	15
ERIC-PCR	ERIC-1: ATGTAAGCTCCTGGGGATTCAC ERIC-2: AAGTAAGTGACTGGGGTGAGCG	52.00	Variable	16

Results

Results of antibiotic resistance. Among all of 70 *E. coli* isolates, the most resistance rates were, respectively, obtained for ampicillin (84.20%), sulfamethoxazole-trimethoprim (65.70%) and cefoxitin (60.00%). APEC isolates demonstrated a high resistance rate to ampicillin (80.00%) and UPEC isolates demonstrated a high resistance rate to ampicillin (88.50%) and sulfamethoxazole-trimethoprim (62.80%). APEC isolates showed the most susceptibility to imipenem and UPEC isolates showed the most susceptibility to gentamycin and ciprofloxacin (Table 2). The percentage of multidrug resistant *E. coli* isolates from APEC and UPEC isolates were 77.10% (27/35) and 68.50% (24/35), respectively. Statistical analysis revealed significant association between APEC and UPEC isolates and antibiotic resistance against ciprofloxacin and imipenem, respectively ($p < 0.05$). Phylogenetic group D showed significant correlation with trimethoprim (in UPEC isolates), cefoxitin, ceftriaxone and sulfamethoxazole-trimethoprim (in APEC isolates).

Table 2. Resistance of the APEC (n =35) and UPEC (n =35) isolates to the different antibiotics.

Isolates	Number of isolates resistant to antibiotics (%)											
	TET	ERY	TMP	GEN	CIP	FOX	CTR	FEP	IMP	SXT	AMP	NAL
APEC	22(62.80)	20(57.10)	18(51.40)	1(2.80)	5(14.20)	23(65.70)	17(48.50)	6(17.10)	0(0.00)	24(68.50)	28(80.00)	21(60.00)
UPEC	17(48.50)	21(60.00)	12(34.20)	2(5.70)	2(5.70)	19(54.20)	15(42.80)	8(22.80)	4(11.40)	22(62.80)	31(88.50)	15(42.80)
Total (%)	39(55.70)	41(58.50)	30(42.80)	3(4.20)	7(10.00)	42(60.00)	32(45.70)	14(20.00)	4(5.70)	46(65.70)	59(84.20)	36(51.40)

TET: tetracycline, ERY: erythromycin, TMP: trimethoprim, GEN: gentamicin, CIP: ciprofloxacin, FOX: cefoxitin, CTR: ceftriaxone, FEP: cefepime, IMP: imipenem, SXT: sulfamethoxazole/trimethoprim, AMP: ampicillin and NAL: nalidixic acid.

Distribution of resistance genes. Total number of 58 resistance gene patterns were observed among 70 *E. coli* isolates. The most prevalent patterns were *dfrA*$^{+}$/*tetA*$^{+}$ and *dfrA*$^{+}$/*bla*$_{CTX\text{-}M}^{+}$ (Table 3). Among all 70 isolates, the most prevalent resistance genes were *dfrA* (48.50%) and *bla*$_{TEM}$ (41.40%), respectively. The *dfrA* (57.10%), *tetA* (45.70%) and *bla*$_{CTX\text{-}M}$ (45.70%) were the most prevalent resistance genes among APEC isolates and the *sul1* (48.50%) was the most prevalent resistance gene among UPEC isolates. Statistical analysis revealed significant difference between APEC and UPEC isolates in association with *qnr* resistance gene ($p < 0.05$). In addition, there were remarkable but non-significant differences between APEC and UPEC isolates associated with *aac*, *tetC* and *bla*$_{CTX\text{-}M}$ genes ($p > 0.05$). The most prevalent pattern of the presence of *tet* genes among tetracycline resistant isolates of APEC and UPEC were *tetA*$^{+}$ and *tetA*$^{+}$/*tetB*$^{+}$, respectively. Among the APEC and UPEC isolates, 65.70% and 51.40% presented ESBL-encoding genes, respectively. *bla*$_{TEM}$ and *bla*$_{CTX\text{-}M}$ were the most prevalent β-lactamase related genes among UPECs and *bla*$_{CTX\text{-}M}$ was the most prevalent β-lactamase gene among APECs. The *sul1* gene was more prevalent among sulfonamide resistant isolates. No significant relationship was observed between the presence of a specific resistance gene and a specific phylogenetic group.

Phylogenetic grouping and DNA fingerprinting. According to phylogenetic grouping 34.20% (24 / 70), 32.80% (23 / 70), 20.00% (14 / 70) and 12.80% (9 / 70) of the isolates were belonged to the A, B2, D and B1 groups, respectively (Table 4). The most prevalent phylogenetic groups among APEC and UPEC isolates were B2 and A with the frequency of 17.00 (48.50%) and 15.00 (42.80%), respectively (Fig. 1). The results of the DNA fingerprinting of the isolates using ERIC-PCR are shown in Figure 1 as a dendrogram associated with phylogenetic groups and resistance gene patterns (Fig. 2).

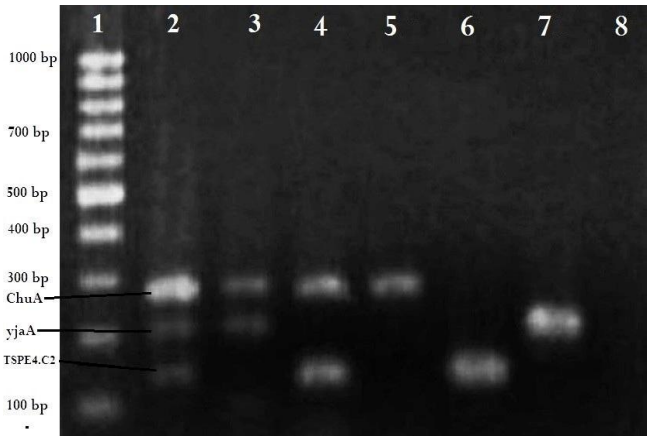

Fig. 1. Multiplex PCR patterns specific for *E. coli* phylogenetic groups. Combination of *chuA, yjaA* and *TSPE4.C2* genes amplification allowed phylogenetic group determination of an *E. coli* isolate. Lane 1: DNA marker, Lanes 2 and 3: Group B2, Lanes 4 and 5: Group D, Lane 6: Group B1: Lanes 7 and 8: Group A.

Table 3. Resistance gene profiles of the APEC and UPEC isolates.

Pattern of resistance genes	Number of isolates		
	APEC	UPEC	Total
Sul2		1	1
dfrA		1	1
tetA	1	1	2
tetB	1		1
***bla*$_{VEB}$**		1	1
***bla*$_{TEM}$**		1	1
Sul1/dfrA		2	2
Sul1/ereA		1	1
Sul2/tetA		1	1
Sul1/tetB		1	1
***Sul2/bla*$_{TEM}$**		1	1
***qnr/bla*$_{VEB}$**	1		1
***qnr/bla*$_{CTX\text{-}M}$**	2		2
dfrA/tetA	3	1	4
***dfrA/bla*$_{TEM}$**	1		1
***dfrA/bla*$_{CTX\text{-}M}$**	3	1	4
***dfrA/bla*$_{VEB}$**	1		1
tetA/tetB	2		2
***tetA/bla*$_{TEM}$**	1		1
***tetB/bla*$_{TEM}$**		1	1
***tetB/bla*$_{CTX\text{-}M}$**	1	1	2
***tetC/bla*$_{VEB}$**		1	1
***tetC/bla*$_{CTX\text{-}M}$**	1		1
Sul1/sul2/dfrA	1		1
Sul1/sul2/ereA		1	1
Sul1/sul2/tetC		1	1
Sul1/dfrA/tetA	1		1
Sul1/dfrA/tetB	1	1	1
***Sul1/dfrA/bla*$_{CTX\text{-}M}$**		2	2
***Sul2/tetA/bla*$_{CTX\text{-}M}$**	1		1
***qnr/bla*$_{TEM}$*/bla*$_{CTX\text{-}M}$**		1	1
***dfrA/tetA/bla*$_{TEM}$**	1		1
***dfrA/ereA/bla*$_{CTX\text{-}M}$**	1		1
***dfrA/bla*$_{TEM}$*/bla*$_{CTX\text{-}M}$**		1	1
***tetA/tetB/bla*$_{TEM}$**		1	1
Sul1/sul2/ereA/aac		1	1
***Sul1/sul2/dfrA/bla*$_{TEM}$**	1		1
Sul1/sul2/dfrA/tetC		1	1
Sul1/sul2/dfrA/tetB	1		1
***Sul1/qnr/tetC/bla*$_{CTX\text{-}M}$**		1	1
***Sul1/dfrA/tetB/bla*$_{CTX\text{-}M}$**	1		1
***Sul1/dfrA/aac/bla*$_{VEB}$**		1	1
Sul1/dfrA/tetA/tetB		1	1
***Sul1/dfrA/tetA/bla*$_{CTX\text{-}M}$**	1		1
***Sul1/qnr/bla*$_{TEM}$*/bla*$_{CTX\text{-}M}$**	1		1
***Sul1/dfrA/bla*$_{TEM}$*/bla*$_{CTX\text{-}M}$**	1		1
Sul1/ereA/tetA/tetB		1	1
***Sul1/ereA/tetC/bla*$_{CTX\text{-}M}$**	1		1
***Sul1/tetA/tetB/bla*$_{ctx\text{-}M}$**		1	1
Sul2/ereA/tetA/tetB	1		1
***Sul2/tetA/tetB/bla*$_{TEM}$**		1	1
***dfrA/tetA/bla*$_{TEM}$*/bla*$_{ctx\text{-}M}$**		1	1
dfrA/ereA/tetA/tetB	1		1
***dfrA/tetA/tetB/bla*$_{ctx\text{-}M}$**	1		1
***Sul1/sul2/dfrA/ereA/bla*$_{TEM}$**	1		1
***Sul1/dfrA/tetA/tetB/bla*$_{TEM}$**	1		1
***qnr/aac/tetA/tetB/bla*$_{CTX\text{-}M}$**	1		1
No resistance gene		1	1

Table 4. Distribution of resistance genes among phylogenetic groups (phylogroups) of APEC, and UPEC isolates.

Isolates	Phylogroups	No.	Resistance gene distribution (%)													Total
			Sul1	*Sul2*	*qnr*	*dfrA*	*ereA*	*aac*	*tetA*	*tetB*	*tetC*	*bla*$_{TEM}$	*bla*$_{VEB}$	*bla*$_{CTX-M}$	*bla*$_{SHV}$	
APEC	A	9	1	1	2	4	0	0	4	4	0	0	1	3	0	29
	B1	3	1	0	1	2	0	0	0	0	0	2	1	1	0	11
	B2	17	6	3	2	10	3	1	9	3	2	4	0	8	0	68
	D	6	2	1	0	4	2	0	3	3	0	1	0	4	0	26
	-	35	10 (28.50)	5 (14.20)	5 (14.20)	20 (57.10)	5 (14.20)	1 (2.80)	16 (45.70)	11 (31.40)	2 (5.70)	7 (20.00)	2 (5.70)	16 (45.70)	0 (0.00)	-
UPEC	A	15	6	4	1	3	3	0	4	4	2	5	2	2	0	51
	B1	6	1	3	0	1	0	1	2	1	0	2	1	0	0	18
	B2	6	4	1	0	3	1	1	1	2	0	1	0	2	0	22
	D	8	6	1	1	7	0	0	2	1	2	1	0	5	0	34
	-	35	17 (48.50)	9 (25.70)	2 (5.70)	14 (40.00)	4 (11.40)	2 (5.70)	9 (25.70)	9 (25.70)	4 (11.40)	9 (25.70)	3 (8.50)	9 (25.70)	0 (0.00)	-
Total		70	27 (38.50)	14 (20.00)	7 (10.00)	34 (48.50)	9 (12.80)	3 (4.20)	25 (35.70)	20 (28.50)	6 (8.50)	16 (22.80)	5 (7.10)	25 (35.70)	0 (0.00)	-

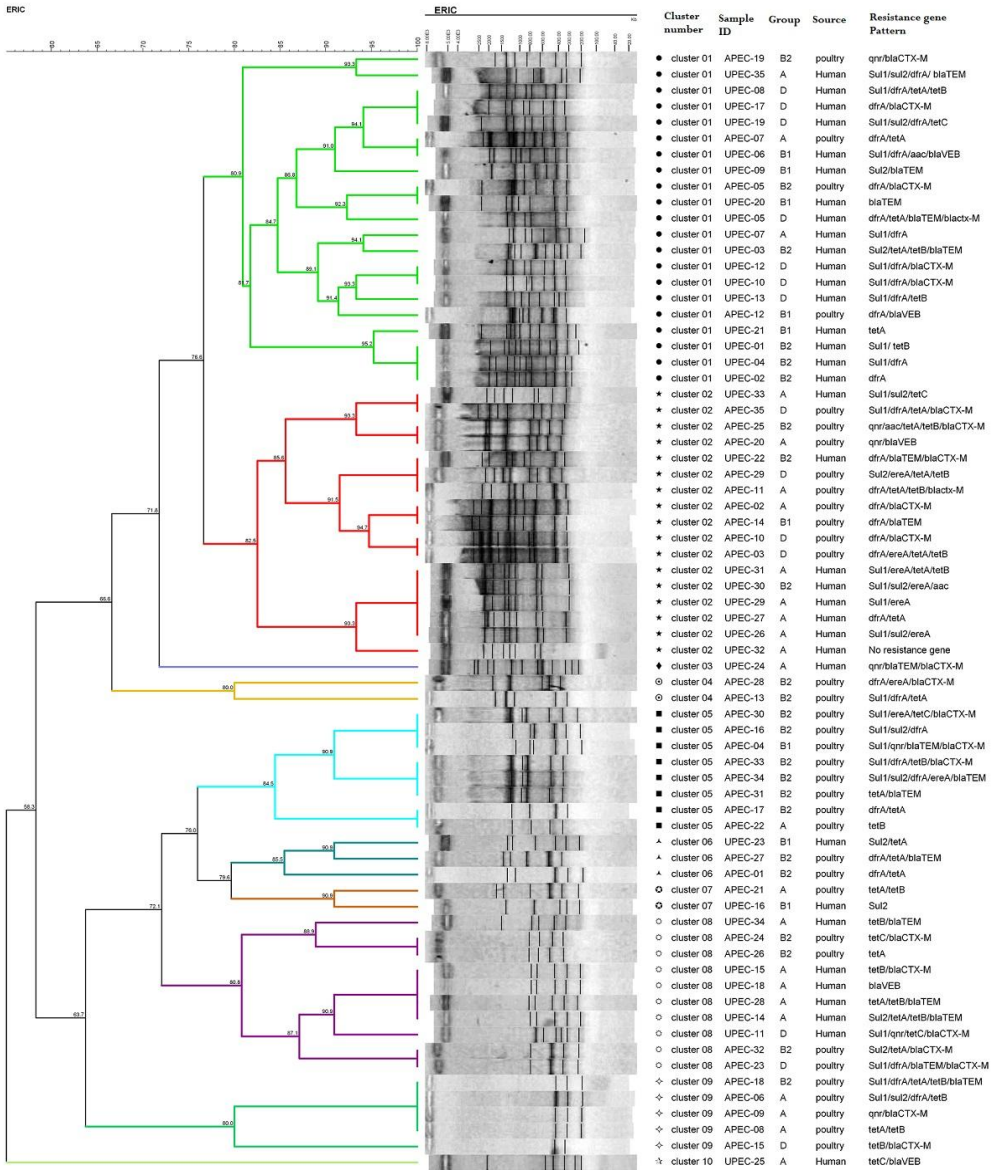

Fig. 2. Dendrogram based on ERIC-PCR fingerprinting of APEC and UPEC isolates collected from poultry and human using the UPGMA analysis. ERIC-PCR assay resulted in 10 different clusters.

Totally, 10 distinct clusters were obtained from analysis of ERIC-PCR results using BioNumerics software (UPMEGA) named C1 to C10 (SID = 0.191). There was no significant correlation or association between phylogenetic groups, resistance gene patterns and ERIC-PCR clusters ($p > 0.05$). The C4, C5 and C9 clusters were observed in particular among APEC isolates, however, C3 and C10 were obtained only in UPEC isolates. The presence of C1 cluster was significant ($p < 0.05$) in correlation with UPEC isolates with the frequency of 17of 35.

Discussion

One of the important issues in investigating the possible similarities of APEC and UPEC strains is the study of resistance behavior of these bacteria against different antibiotics as well as phylogenetic differences. The first remarkable result of the present study was the similarity between APEC and UPEC isolates in the type of the antibiotic with the highest resistance, ampicillin and sulfamethoxazole-trimethoprim. However, among studied antibiotics, APEC and UPEC isolates showed significant difference in resistance to ciprofloxacin and imipenem, respectively. Use of the different antibiotics in human and poultry associated *E. coli* infections can be one of the causes of this phenomenon. In poultry farms of some countries such as Iran, fluoroquinolone antibiotics such as enrofloxacin were the first approach to fight against AC,[17,18] regardless of the fact that this indiscriminate use could cause resistance to other antibiotics in this drug category such as ciprofloxacin that were used in the treatment of human infections. On the other hand, carbapenems are widely used in gram-negative human infections,[19] and more resistance among human isolates is expected. Gentamycin, imipenem and ciprofloxacin had proper antibacterial effect on *E. coli* isolates in the present study and it was in accordance with other studies that reported high susceptibility of the APEC and UPEC isolates to these agents.[5,20,21] However, the prevalence of the MDR isolates did not show significant difference between these two pathotypes of *E. coli*. This relatively high level of MDR isolates could be feedbacked from the indiscriminate and unsupervised use of antibiotics in veterinary medicine and even self-medications in human infections.[22,23]

In terms of the presence of resistance genes among APEC and UPEC isolates, high diversity was observed in obtained patterns. Conceivably, the use of diverse antibiotics in human infections and treatment of avian colibacillosis in poultry flocks has led to the survival of resistant strains with various plasmid or / and transposon resistance genes. In addition, according to the results, the number of APEC isolates with at least 4 resistance genes is higher than UPEC isolates. Particularly, CTX-M producing isolates which have been increased during recent decades and plays an important role in β-lactamase resistance.[24] In our study, $bla_{CTX\text{-}M}$ was found as the predominant gene among ESBL associated genes of *E. coli* isolates while none of the isolates showed the presence of the bla_{SHV} gene in contradictory to some other.[24-26] The investigation of Durmaz *et al.* showed that the ratio of the $bla_{CTX\text{-}M}$ gene as the predominant type of beta-lactamases was very high at 93.00% whereas the bla_{SHV} and bla_{TEM} genes were 65.00% and 49.00%, respectively.[27] Kim *et al*. showed that the *sul2*, *tetA* and bla_{TEM} were the most prevalent genes among APEC isolates, respectively, for sulfonamides, tetracycline and beta-lactamases in South Korea.[5] Other studies reported the importance of the *sul1*, $bla_{CTX\text{-}M}$ and *tetB* genes in resistance of the *E. coli* isolates in accordance with the results of the present study.[24,26,28]

The dominant phylogenetic group was different among the isolates of APEC and UPEC (B2 for APECs and A for UPECs) in the present study and in examining the ratio of the frequency of resistance genes to the quantity of each phylogenetic group, this ratio in group D was achieved more than other groups. Zenati *et al*. also showed A type as the predominant phylogenetic group among UPEC isolates,[29] while other studies showed the B2 and A as the main phylogenetic groups among UPECs and APECs respectively.[30,31] Different studies used ERIC-PCR for fingerprinting *E. coli* isolates especially obtained from UTI cases in human and presented various numbers of ERIC patterns or clusters representing the variability and heterogeneity of the isolates. Pourakbari *et al*. in a study on 102 *E. coli* strains isolated from UTIs in children showed 13 various ERIC clusters.[32] In other study, ou of 83 *E. coli* isolated from hospitalized patients 14 clusters were observed.[29] Finally, APEC and UPEC isolates displayed heterogeneous and various antibiotic resistance and some phylogenetic groups of *E. coli* isolates showed significant resistance to certain antibiotics, however, phylogenetic grouping and ERIC-PCR based fingerprinting did not show obvious differences between isolates obtained from two different sources. In conclusion, APEC and UPEC isolates in this study showed differences and similarities in the occurrence of resistance genes, however, the answer to the question about the potential risk and hazard of APEC strains for the incidence of disease in humans beyond resistance properties depends on the study of virulence genes and related factors in the pathogenicity of these bacteria.

Acknowledgment

This study was supported by Islamic Azad University of Ayatollah Amoli Branch and Amol University of Special Modern Technologies, Amol, Iran.

Conflict of interest

The authors have no conflict of interest.

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