Extra-intestinal pathogenic *Escherichia coli* from human and avian origin: Detection of the most common virulence-encoding genes

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Abstract

Pathogenic *Escherichia coli* strains cause a wide range of extra intestinal infections including urinary tract infection in humans and colibacillosis in poultry. They are classified into uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC) with genetic similarities and variations. Their pathogenicity is related to the virulence-encoding genes like *sfa*, *papG II*, *ompT*, *iutA*, and *iss* with zoonotic potentials. One hundred isolated *E. coli* from patients with urinary tract infection and 100 *E. coli* from chickens with colibacillosis were evaluated for the presence of the most common virulence-encoding genes including *sfa*, *papG II*, *ompT*, *iutA*, and *iss* by multiplex polymerase chain reaction. While the frequency of *sfa*, *papG II*, *ompT*, *iutA*, and *iss* encoding genes in APEC isolates were respectively 0.00%, 67.00%, 63.00%, 89.00% and 89.00%, the frequency of these encoding genes in UPEC isolates were 18.00%, 40.00%, 40.00%, 74.00% and 48.00%, respectively. Except for *sfa*, the frequencies of other encoding genes in APEC were more than those in UPEC isolates. The *iutA* as the most common UPEC encoding gene and *iss* as the most common APEC encoding gene were the most prevalent virulence factors in the examined *E. coli* isolates. Finding out the distribution of virulence-associated genes could be helpful to identify similarities and differences between APEC and UPEC isolates in order to provide more substantial evidence of their common virulence traits and potential zoonotic threats.

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Introduction

Escherichia coli is the most significant Gram-negative facultative anaerobic bacterium with a wide range of pathogenicity and genotypic diversity.\(^1\) It is a commensal bacterium in the gut of humans and other vertebrates and is responsible for a wide range of intestinal and extraintestinal infections.\(^2\) Accordingly, it is divided into two pathotypes of intestinal pathogenic E. coli and extra-intestinal pathogen E. coli (ExPEC). Each pathotype is divided into sub-pathotypes based on certain common traits of host specificity, pathogenesis mechanism, and infected organ.\(^3\) For example, serogroups named O18 with large virulence plasmids and K1 capsular antigen cause meningitis.\(^4,6\) It is originated from human neonates and named as neonatal-meningitis E. coli. Human uropathogenic E. coli (UPEC) lacking virulence plasmids tends to be of the O2 and O6 serogroups.\(^6\) Similarly, avian pathogenic E. coli (APEC), responsible for poultry colisepticaemia, possesses large virulence plasmids and great diversity in serogroups like O1, O2, and O78.\(^7,8\)

Urinary tract infections (UTIs) are among one of the highest medical costs bacterial infections affecting healthcare, with 150 million cases occurring annually worldwide.\(^9\) The virulence-associated factors involved in the establishment of UTIs enable them to survive and invade noxiously to the host tissues, induce the inflammatory response via disruption of the defense mechanisms and finally cause a variety of diseases even out of the urinary tract.\(^10\) Another virulence trait of this organism is due to the expression of a wide spectrum of antimicrobial resistance genes.\(^11\)

Avian pathogenic E. coli induces multi-target infection in broilers called colibacillosis with large losses to the poultry industry. Its disseminated infection induces fibrinous lesions in internal organs from air sacs and pericardium to peritonea associated with septicemia.\(^12,2,3,13\)

According to the relationship between UPEC and APEC genes for virulence and pathogenicity, the zoonotic risk of isolated avian E. coli should be considered.\(^14,15\) Despite the variable virulence gene profile engaged in the pathogenicity of APEC and human ExPEC, the similarities between these two strains may also share a common ancestor.\(^17\) A lot of studies were done to find serogroup similarity and link between genotype of virulence factors between APEC and UPEC.\(^18\) Common reported virulence-associated genes between APEC and UPEC include iron-limited urinary tract (iutA) related to iron acquisition systems, increased serum survival (iss), S-fimbrial adhesion (sfa), pyelonephritis-associated pil (papG II) and outer-membrane protein T (ompT) genes showing their common traits.\(^10\) Additionally, the prevalence of wide serological diversity was revealed in several studies with particular combinations of virulence-associated genes among APEC strains.\(^6,8,19\) Different virulence assays were mentioned in APEC with various similarities to the UPEC. The most prevalent genes in E. coli isolated from poultry colibacillosis in Iran were hly F, ompT, iss, iutA and pap G II.\(^20\) While tsh, iss, astA, iucD, vat, and papC were the most frequent virulence genes isolated from APEC, other genes including fim, aaadA1 and qnr have been reported as the most virulence genes isolated from UPEC.\(^21,22\) Likewise, the clonal relations between some APEC and human ExPEC strains have been demonstrated.\(^23,25\) Also, serotype O18:K1:H7 isolated from avian has been reported to have pathogenicity for human.\(^26\)

Epidemiological studies have showed genetic similarity and variation between E. coli isolated from poultry and human.\(^27,28\) In order to get distribution of virulence-associated genes in UPEC and APEC strains, we gathered a relatively large collection of them and made a systemic comparison between the prevalence of their virulence-encoding genes. The aim of this study was to find out the distribution of virulence-associated genes to identify the similarities and differences between these groups in order to provide more substantial evidence of their common virulence traits and potential zoonotic threats.

Materials and Methods

Sampling and bacterial culture. A total of 200 E. coli isolates were studied in this survey. They were isolated from the pericardium of broiler chickens suffering or died of colibacillosis (n = 100) and from the urine samples of the patients suffering from urinary infection (n = 100). The urine isolates were collected from the patients having at least 10\(^5\) colonies forming unit of a bacterium per milliliter of urine. The isolates were routinely grown in eosin methylene blue agar (Merck, Darmstadt, Germany). After confirming the bacteria like E. coli by biochemical tests, each one was incubated in trypticase soy broth (TSB; Merck) for 18 hr at 37 °C. Then, each culture was centrifuged at 3000 g for 15 min. After discarding the supernatant, 1.00 µL of the bacterial pellet with 1.00 µL of glycerol and 9.00 µL of TSB were cast in a microtube and stored at –20 °C for the following steps.

DNA extraction. The bacterial samples were mixed with lysis buffer (Bio-Rad, Watford, UK) and incubated for 10 min in a water bath at 55 °C. Protease K (Sigma-Aldrich Co. Ltd., Dorset, UK) was added into each micro tube, vortexed carefully and incubated at 55 °C for 20 min. In the next step, binding buffer was added and incubation was done again at 70 °C for 10 min. After adding pure ethanol (Merck) and vortexing, the microtubes were then centrifuged at 8000 g for 1 min. Briefly, by using washing buffer and then centrifuging them, the ethanol was cleaned up. Finally, the elution buffer was used followed by multistage centrifuge to purify the extracted DNA.

Gene amplification. The E. coli specific gene uidA and virulence-associated genes including sfa, papG II, ompT,
*iutA* and *iss* were detected by multiplex polymerase chain reaction (PCR) assay. The oligonucleotide sequences of primers used for amplifications of the target genes are shown in Table 1. In PCR run, two virulence genes were amplified in a 30.00 µL reaction mixture. The mixture was included Taq DNA Polymerase Master Mix (12.50 µL), Ampliqon (Odense, Denmark), template DNA (8.00 µL) and each 2.00 µL of primes (Metabion international AG, Planegg, Germany) supplemented with 5.50 µL distilled water. The pure *E. coli* culture (ATCC 10536) harboring target genes was obtained from Tehran University, Tehran, Iran and used as a positive control and the mixture of Taq DNA Polymerase Master Mix and distilled water was used as a negative control. Each tube reaction mixture was subjected to denaturation (94 ℃ for 30 sec), annealing (58 ℃ for 30 sec) and extension (72 ℃ for 90 sec), followed by one cycle consisting of 5 min at 72 ℃ in a thermal cycler (ABI 2720; Applied Biosystems, Vilnius, Lithuania). After gene amplification, every PCR product was run on horizontal gel electrophoresis with 2.00% agarose gel, stained with SYBER safe (SinaClon, Tehran, Iran) and illuminated by ultraviolet exposure.

**Statistical analysis.** In order to compare the frequency of virulence genes, Chi-square test and to investigate the relationship between the frequency of genes, Chi-square test, and Spearman correlation index were used. All analyses were performed with SPSS (version 18.0; SPSS Inc., Chicago, USA) and *p* value < 0.05 was the threshold for significance.

**Table 1.** The sequence of primers used for amplifications of *uidA, sfa, papG II, ompT, iutA,* and *iss* encoding genes.

| Genes     | Oligonucleotide sequence | Expected size (bp) | Reference |
|-----------|--------------------------|--------------------|-----------|
| *uidA*    | F: tgtaattacgccagaaagacctgc | 147                | 29        |
|           | R: acgctggcttacctgc      |                    |           |
| *sfa*     | F: ctctgagaaacggatctctac | 410                | 10        |
|           | R: cgcgagagtaaaccctg     |                    |           |
| *papG II* | F: gcctggtgaggcccttggt   | 190                | 10        |
|           | R: cgccggctcaagatagctg   |                    |           |
| *ompT*    | F: atctagcggagaggaagcc   | 559                | 10        |
|           | R: cccggctatagtgttcatc   |                    |           |
| *iutA*    | F: ggtcgagcatgaggaagttc  | 302                | 10        |
|           | R: cgtggagaacggtagataagc |                    |           |
| *iss*     | F: cggctgcggacgtgccgagaa | 323                | 10        |
|           | R: agcgacgccgagacggccgaa |                    |           |

**Results**

Figure 1 shows the electrophoresis of the PCR products. While the isolates from poultry colibacillosis (P1-P7) and human UTIs (H1-H7) were *uidA* positive, the samples of P2-P7, H1, H2, H6, and H7 were also *ompT* positive (Fig. 1A). The P1-P6, P8, P10-P14, H1-H4, H7, H8, and H11-H13 isolates were positive for the *iutA* gene (Fig. 1B). While H1 and H8 contained *sfa*, none of the isolated strains from poultry colibacillosis contained this virulence gene.

The electrophoresis of genes products of *iss* and *papG II* from colibacillosis and human UTIs is shown in Figure 1C. The isolates P1, P2, P6-P14, H1-H10, and H12 contained *iss* gene. The *papG II* was present in P2-P5, P8, H5-H7, H9, and H10 isolates.

The frequency of the *sfa, pap GII, ompT, iutA* and *iss* genes in *E. coli* strains isolated from APEC and UPEC is shown in Table 2. Except for *sfa* gene, the frequency of other genes from APEC was significantly higher than that of UPEC (*p* < 0.05). According to Table 3 summarizing the virulence-encoding genes in APEC and UPEC, most isolates of APEC and UPEC had three (40.00%) and two (44.00%) virulence-encoding genes, respectively. None of them had all five virulence-encoding genes.

**Table 2.** The frequency (%) of virulence encoding genes in avian pathogenic *E. coli* (*n* = 100) and human uropathogenic *E. coli* (*n* = 100).

| Genes     | APEC1 isolates | UPEC2 isolates | *p* value |
|-----------|----------------|----------------|-----------|
| *sfa*     | 0              | 18             | 0.000*    |
| *papG II* | 67             | 40             | 0.000*    |
| *ompT*    | 63             | 40             | 0.001*    |
| *iutA*    | 89             | 74             | 0.006*    |
| *iss*     | 89             | 48             | 0.000*    |

* indicates significant differences between APEC and UPEC isolates at *p* < 0.001.

1Avian pathogenic *Escherichia coli*; 2 Human uropathogenic *E. coli*. 
Table 3. The frequency of avian pathogenic E. coli (n = 100) and human uropathogenic E. coli (n = 100) in terms of the number of virulence encoding genes.

| Isolates | Number of genes | Frequency (%) |
|----------|-----------------|---------------|
|          | 0  | 1  | 2  | 3  | 4  | 5  |          |
| APEC1    | 1  | 4  | 18 | 40 | 37 | 0  |          |
| UPEC2    | 2  | 3  | 6  | 12 | 6  | 0  |          |

1Avian pathogenic Escherichia coli; 2Human uropathogenic E. coli.

Table 4 shows the simultaneous presence of two virulence-encoding genes in APEC and UPEC isolates. The correlation between the frequency of virulence-encoding genes between APEC and UPEC isolates is also listed. While in UPEC isolates, between sfa, iss, iutA, iss and papG II a significant negative and between sfa and papG II a significant positive correlation was observed, no correlation was seen between any of these genes in APEC isolates.

In Table 5, the results of the simultaneous presence of three and four genes in APEC and UPEC isolates are shown. Fifty-three percent of APEC isolates had iutA, and papG II and 53.00% percent of APEC isolates had also iutA, ompT and iutA simultaneously. While 39.00% of APEC isolates had iutA, papG II and ompT together and 41.00% had iss, ompT, and papG II simultaneously, in UPEC isolates only one or two percent of strains had these genes together. Thirty-seven percent of UPEC isolates had four genes of papG II, iutA, ompT and iss in common.

Discussion

Avian pathologic E. coli is one of the most bacterial cause of infectious diseases in poultry and the main cause of poultry colibacillosis. This infection is responsible for great losses in the poultry industry.1,2,3,30 Recently, the zoonotic potential of APEC strains was considered by some researchers.19,31 In several studies, the phylogenetic, genotypic and serotype relation between APEC strains and extra-intestinal E. coli strain in human, like UPEC, has been demonstrated. Similar virulence factors with the same mechanism between APEC and UPEC strains have been concluded.10,26,32

Table 4. The frequency (%) of avian pathogenic E. coli (n = 100) and human uropathogenic E. coli (n = 100) in terms of carrying two virulence encoding genes.

| Genes           | APEC1 Spearman correlation | p value | UPEC2 Spearman correlation | p value |
|-----------------|----------------------------|---------|----------------------------|---------|
| ompT, iss       | 57                         | 0.03    | 0.70                       |         |
| papG II, iss    | 60                         | 0.06    | 0.50                       |         |
| iutA, iss       | 80                         | 0.06    | 0.50                       |         |
| iss, sfa        | 0                          | -       | -                          |         |
| sfa, ompT       | 0                          | -       | -                          |         |
| sfa, papG II    | 0                          | -       | -                          |         |
| sfa, iutA       | 0                          | -       | -                          |         |
| ompT, papG II   | 23                         | 0.10    | 0.10                       |         |
| ompT, iutA      | 62                         | 0.03    | 0.70                       |         |
| papG II, iutA   | 60                         | 0.00    | 1.00                       |         |

††† indicate significant differences between APEC and UPEC isolates at p < 0.05, p < 0.001, respectively

1Avian pathogenic Escherichia coli; 2Human uropathogenic E. coli.

In the present study, we evaluated the frequency of sfa, papG II, ompT, iutA and iss as virulence-encoding genes of E. coli and identified four genes including papG II, ompT, iutA, and iss in both APEC and UPEC. The similar result was reported previously.10 These results evidently have concluded the relation between APEC and UPEC and confirmed the previous knowledge of the role of certain genes specified to a particular host (human versus avian and/or urinary tract versus respiratory tract).33,34 The frequency of virulence-related genes in APEC including sfa, papG II, ompT, iutA and iss in our study was respectively 0.00%, 67.00%, 63.00%, 89.00% and 89.00% versus 2.00%, 43.00%, 60.00%, 90.00% and 81.00% in the report of Zhao et al.10 In another study, iutA, iss and papG II genes had the frequencies of 50.00%, 40.00% and 15.20%, respectively.35 It was also reported that more than 97.00% of APEC had sfa and fim virulence-encoding genes.26 In our study, none of the APEC isolates had sfa, which is in agreement with Zhao et al.10 and is in contrast with Moulin-Schouleur et al.26 Among the genes examined for this study, iutA, and iss as virulence factors encoding genes were detected in the
The positive correlation indicates that by changing the frequency of gene encoding virulence adhesion factors, sfa, the frequency of the gene encoding adhesion factor papG II was also increased. But, none of the virulence genes in APEC showed a significant correlation, which means that change in the frequency of none of the mentioned genes affects each other.

Generally, the results of the present study indicated the relation of genes encoding virulence factors between human and poultry and showed that both APEC and UPEC isolates encounter similar challenges in establishing infection. These results indicate the zoonotic risk of APEC. Therefore, further studies have to be done in poultry products such as chicken meat and eggs. However, due to the limitation of the present study in terms of lack of solid phylogenetic linkage between APEC and UPEC strains, the number of virulence-associated genes and investigating the ability of APEC to get passed the body’s defenses to cause UTIs in humans can be considered in future studies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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