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# Prevalence of Toxigenic Genes in *Escherichia Coli* Isolates From Hospitalized Patients in Zabol, Iran

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**Abstract**

**Background:** Uropathogenic *Escherichia coli* (UPEC) are a common causative agent of urinary tract infections. Strains of UPEC encode a number of virulence factors that facilitate their dissemination and persistence within the host. To diminish the burden of UPEC, using effective preventive measures, data on virulence factor prevalence in different geographic regions must be assessed.

**Objectives:** As no such data was available for this geographic region of Iran, the purpose of this study was to analyze the prevalence of ten UPEC virulence genes among 100 *E. coli* isolates collected from patients with urinary tract infections (UTI) in Zabol, Iran.

**Patients and Methods:** One hundred UPEC obtained from patients with urinary tract infection were screened by the polymerase chain reaction (PCR) with primers specific for the following UPEC virulence genes: *astA* (enterotoxins), *cdtB* (enterotoxins), *cvi/cva* (colicin V operon), *ibeA* (an invasive protein), *iss* (increased serum survival protein), *iutA* (aerobactin), *kpsII* (group 2 capsule), *neuS* (K1 polysialyltransferase), *tsh* (an adhesive and proteolytic protein), and *vat* (vacuolating autotransporter toxin).

**Results:** Amongst the total of 100 UPEC isolates, 99 (99%) isolates were found to carry the studied virulence genes. Twenty-six different virulence patterns were identified. The prevalence of *astA*, *cdtB*, *cvi/cva*, *ibeA*, *iss*, *iutA*, *kpsII*, *neuS*, *tsh* and *vat* were 29%, 0%, 19%, 67%, 47%, 99%, 98% 96%, 1% and 18%, respectively.

**Conclusions:** We concluded that major differences exist in the prevalence of virulence factors between different UPEC isolated from different countries. Detecting these genes as primary controllers of UPEC virulence may aid in better management of related infections.

*Keywords:* Uropathogenic *Escherichia Coli*, Toxin Gene, Virulence Factors

## 1. Background

Uropathogenic *Escherichia coli* (UPEC) are the major causative agent of Urinary Tract Infections (UTI) (1). They contain several virulence factors that facilitate their colonization and invasion of host cells (2, 3) Besides other factors, toxins, adhesion and invasion are amongst the most important virulence factors in a variety of *E. coli*-mediated diseases. Production of toxins by colonizing *E. coli* may cause an inflammatory response, a possible pathway for UTI symptoms. The most important secreted virulence factors of UPEC are *astA* (enterotoxins) (4), *cdtB* (enterotoxins) (5), *cvi/cva* (colicin V operon) (6), *ibeA* (an invasive protein) and *iutA* (aerobactin) (7, 8), *iss* (increased serum survival protein) (9), *kpsII* (group 2 capsule) (10), *neuS* (K1 polysialyltransferase) (11), *tsh* (an adhesive and proteolytic protein) (12), and *vat* (vacuolating autotransporter toxin) (13, 14). These toxins are molecules that are secreted on the bacterial cell surface, or proteins that are released into the external environment, enabling UPEC to quickly multiply and infect their hosts. Apart from syndromes caused by toxin production, UPEC pathogenesis results from synergistic interactions of a variety of the above-mentioned factors. Furthermore, UPEC strains fall under the category of extraintestinal pathogenic *E. coli*, which are characterized by the possession of virulence factors that enable them to live an extraintestinal life (15, 16). Previous studies have shown a large diversity in the distribution of virulence factors among UPEC strains (1, 17-19). However, no specific virulence factor that contributes entirely to the pathogenicity of UPEC has been discovered (20, 21). Thus, a lack of adequate diagnostic tests, to determine degrees of the virulence, makes it difficult to control urinary tract infections (22). In addition, the lack of diagnostic tests also results in scarce knowledge about the epidemiology of UPEC.

## 2. Objectives

The aim of this study was to screen 100 UPEC isolates at the teaching Hospital of Zabol, for the presence of different virulence factors, and to analyze the presence of such factors, alone or in combination, in order to evaluate the

epidemiological prevalence of UPEC in Zabol by the multiplex PCR.

## 3. Patients and Methods

### 3.1. Isolation and Identification of Uropathogenic Escherichia Coli Isolates

A total 100 UPEC isolates were identified by conventional biochemical and morphological tests during January to July 2013, as described previously by our group and Kalantar et al. (23, 24). The inclusion criteria were referral to the teaching hospital and presenting symptoms of urinary tract infection. The exclusion criterion was having received antibiotic therapy within one week before sampling. Ethical approval was granted by the Iranian Ministry of Health and Medical Education and the city's local research ethics committee. The personal information and patient's medical records were recorded and kept strictly confidential.

### 3.2. DNA Extraction

All *E. coli* isolates were grown overnight (16 hours) in 5 mL luria bertani (LB) broth in a shaking incubator (200 rpm) at 37°C. Two milliliters of bacterial culture broth were then pelleted, resuspended in 200 µl of sterile double-distilled water and boiled at 95°C for 10 minutes. The suspension was then chilled on ice for five minutes, and the supernatant was collected after centrifugation at 13000 rpm for five minutes. After centrifugation, the supernatants were stored at -20°C until use for the PCR.

### 3.3. Multiplex Polymerase Chain Reaction

In the present study the most important virulence genes of UPEC strains including *astA*, *cdtB*, *cvi*/*cva*, *ibeA*, *iss*, *iutA*, *kpsII*, *neuS*, *tsh* and *vat* were detected in two separate PCR reactions comprised of five forward and reverse primers. Primers were designed using Mpprimer (http://biocompute.bmi.ac.cn/MPprimer/) (Table 1). The Multiplex-PCR method was performed with a total volume of 25 µL including 12.5 µL Taq DNA polymerase master mix red (amplicon), 0.2 µM/µL of each primer (1 µL) (Pishgam, Iran), 2 µL (approx imately 100 ng/µL) of genomic DNA, and 9.5 µL dd $H_2O$. The DNA was then amplified by 30 successive cycles of denaturation at 95°C for 40 seconds, primer annealing at 57°C for 30 seconds, and DNA chain extension at 72°C for 30 seconds with a programmable gradient Eppendorf's Master cycler® pro (Eppendorf, Hamburg, Germany). All PCR products were analyzed by electrophoresis (85V) in 1.5% agarose gel, stained by ethidium bromide and imaged with a GelDoc 1000 (Vilber Lourmat, France) image analysis station. A molecular weight marker with 100 bp increments (100 bp ladder, Fermentas, Germany) was used as the size standard.

**Table 1.** Primer Sequence Used in This Study

| Primer Name | Sequence (5' - 3') | Length (bp) |
|---|---|---|
| ***astA*** | | 116 |
| F | TGCCATCAACACAGTATATCC | |
| R | TCAGGTCGCGAGTGACGGC | |
| ***iss*** | | 309 |
| F | ATCACATAGGATTCTGCCG | |
| R | CAGCGGAGTATAGATGCCA | |
| ***vat*** | | 981 |
| F | TCCTGGGACATAATGGTCAG | |
| R | GTGTCAGAACGGAATTGT | |
| ***Cvi/Cva*** | | 514 |
| F | TCTGTTGGCTCGGTTATTAG | |
| R | CAAAACCACAAAAGCCTCTC | |
| ***tsh*** | | 824 |
| F | ACTATTCTCTGCAGGAAGTC | |
| R | CTTCCGATGTTCTGAACGT | |
| ***neuS*** | | 489 |
| F | GGCCTGCCAGAACTGGTG-CAAA | |
| R | CGTGCATACTGGCGCAAGCAAC | |
| ***iutA*** | | 174 |
| F | ACCGACAGCCGACAACTGGACT | |
| R | GCCACTTTTGGTGCCAGCCTCA | |
| ***Kps II*** | | 253 |
| F | TCAGCCGCTCACCGATTCCGTA | |
| R | ATCAGGGCGCGCACTTTTTCCA | |
| ***ibeA*** | | 377 |
| F | AACGTTGTCAGCATCCCTGCCG | |
| R | ATTGCCCGTCCGCAAACCAACA | |
| ***cdtB*** | | 585 |
| F | GCCGCGGAGCTGGTTCATCATT | |
| R | TGAAGGTTCCAGGTCGCAACGC | |

## 4. Results

### 4.1. Multiplex Polymerase Chain Reaction

The distribution of selected virulence genes in the UPEC isolates is shown in Figure 1. We found that 99% of the isolates were positive for at least one virulence gene. Table 2 shows that one isolate harboured two genes simultaneously, ten isolates harboured three genes, and in twenty eight isolates four genes were detected. Thirty-nine isolates possessed five virulence genes. There was no strain that contained all 10 of the virulence-associated genes. However, nine isolates possessed six genes,

while ten isolates had seven of the genes. Patterns and combinations of virulence-associated genes for 100 isolates collected in the present study are summarized in Table 2. Also, the prevalence of *iutA*, *kpsII*, *neus*, *ibeA* and *iss* virulence factors of UPEC isolates were 99, 98, 96, 67 and 47%, respectively, while the presence of *astA*, *vat*, cvi-cva and *tsh* had lower frequencies (29, 18, 19 and 1%, respectively) (Figure 1). Overall, *iutA*, *kps II* and *neuS* were the most commonly detected putative virulence genes in UPEC isolates. The results showed that there were no positive samples for *cdtB* virulence gene of *E. coli* isolated from patients with UTIs. Based on the distribution of the various targeted sequences, all the studied strains exhibited 26 virulence gene patterns, where each pattern was given a reference number proceeding the designation *Escherichia coli* (Ec) (Table 2).

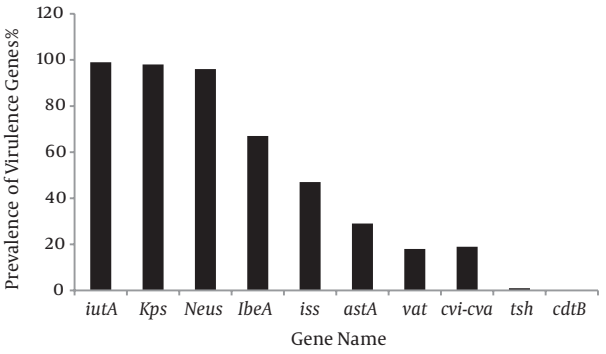


**Figure 1.** Prevalence of Genes Encoding Virulence Factors Among One Hundred Uropathogenic *Escherichia Coli* Isolates

**Table 2.** Virulence Patterns of the Studied Isolates

| Patterns | Virulence Genes | | | | | | | | | | N of Isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *iutA* | *kps II* | *neuS* | *ibeA* | *iss* | *astA* | *Cvi/cva* | *vat* | *tsh* | *cdtB* | |
| **EC 1** | + | + | + | + | + | + | + | NA | NA | NA | 4 |
| **EC 2** | + | + | + | + | + | + | NA | + | NA | NA | 2 |
| **EC 3** | + | + | + | + | + | NA | + | + | NA | NA | 3 |
| **EC 4** | + | + | + | + | + | NA | + | NA | + | NA | 1 |
| **EC 5** | + | + | + | + | + | NA | NA | + | NA | NA | 3 |
| **EC 6** | + | + | + | NA | + | NA | + | + | NA | NA | 2 |
| **EC 7** | + | + | + | NA | + | + | NA | + | NA | NA | 2 |
| **EC 8** | + | + | + | + | + | + | NA | NA | NA | NA | 1 |
| **EC 9** | + | + | + | + | + | NA | + | NA | NA | NA | 2 |
| **EC 10** | + | + | + | NA | + | + | + | NA | NA | NA | 1 |
| **EC 11** | + | + | + | NA | + | + | NA | NA | NA | NA | 4 |
| **EC 12** | + | + | + | NA | NA | NA | + | + | NA | NA | 1 |
| **EC 13** | + | + | + | + | NA | + | NA | NA | NA | NA | 8 |
| **EC 14** | + | + | + | + | + | NA | NA | NA | NA | NA | 15 |
| **EC 15** | + | + | + | + | NA | NA | NA | + | NA | NA | 7 |
| **EC 16** | + | + | + | + | NA | NA | + | NA | NA | NA | 3 |
| **EC 17** | + | + | + | NA | + | NA | + | NA | NA | NA | 1 |
| **EC 18** | + | + | + | NA | NA | + | NA | NA | NA | NA | 5 |
| **EC 19** | + | + | NA | NA | + | + | NA | NA | NA | NA | 1 |
| **EC 20** | + | + | + | + | NA | NA | NA | NA | NA | NA | 18 |
| **EC 21** | + | + | + | NA | + | NA | NA | NA | NA | NA | 3 |
| **EC 22** | + | + | + | NA | NA | NA | + | NA | NA | NA | 1 |
| **EC 23** | + | + | + | NA | NA | NA | NA | NA | NA | NA | 9 |
| **EC 24** | + | NA | NA | NA | + | + | NA | NA | NA | NA | 1 |
| **EC 25** | + | + | NA | NA | NA | NA | NA | NA | NA | NA | 1 |
| **EC 26** | NA | NA | NA | NA | + | NA | NA | NA | NA | NA | 1 |
| **Total** | 99 | 98 | 96 | 67 | 47 | 29 | 19 | 18 | 1 | 0 | 100 |

Abbreviations: +, Positive for Virulence Genes; NA, Not Available.

## 5. Discussion

The repertoire of virulence genes present in a certain strain and determines the severity of disease (25). Toxins, adhesion and invasion are well established virulence factors that are often responsible for the major symptoms of a bacterial infection (1). The prevalence of the detected genes among the studied isolates and the frequency of concurrent detection of various virulence factors are summarized in Figure 1. While no copy of the *cdtB* gene was detected in any of the isolates, a relatively high number of gene pattern combinations were observed (Table 2). Our results showed a lower prevalence of *astA* and *vat* in UPEC isolates compared to other parts of Iran, which indicates the geographical distribution of virulence factors (26, 27). The iron uptake systems are also present in UPEC, and some strains have developed more than one strategy for sequestering iron from their hosts (28).

In this study, we examined one gene associated with iron uptake. In addition to the *iroN, iucD* and *irp2* gene, which were previously discussed by our group (17, 29, 30), we studied the *iutA* gene, which was found in 99% of the isolates. The high prevalence of *iutA* indicates a certain role for such gene in iron uptake. This prevalence is in agreement with epidemiological surveys performed by Blanco (94%) (31) and Johnson (60%) (32). The increasing occurrence of K-antigen polysaccharide (KPS) among the UPEC indicates a progressive accumulation of K-positive *E. coli* strains along the course of urinary tract infections (UTI). Since the KPS antigen is known for its serum resistance, antiphagocytosis, the selection of UPEC isolates carrying such pathogenic trait by the urinary tract ecosystem could play an important role in extraintestinal pathogenesis. In the present study, the prevalence of genes coding for capsular polysaccharide, *neus* and kps, among clinical isolates were 96% and 98%, respectively; this is the first report on such genes from our country with regards to UPEC isolates. Moreover, our results show that *neus* and kps genes have a potential value for selection as a candidate antigen for designing a new vaccine against UTI.

Frequencies of isolates with *ibeA*, a gene associated with invasion, was 67%. Various studies investigated the prevalence of invasion encoding genes in *E. coli* isolates from patients with UTI (17, 30).

A study by Lopez-Banda, in contrary to our study showed that almost 3% of *E. coli* isolates were positive for the *ibeA* gene (3% versus 67%) (33). The gene associated with invasion, *ibeA*, is generally present among avian pathogenic *Escherichia coli* (APEC) (34) and neonatal meningitis-causing *Escherichia coli* (NMEC) (35) strains. These differences may be due to the differences in geographical regions. In our study, the prevalence of genes coding for iron uptake systems, capsular polysaccharide and invasion was high while the presence of toxin encoding genes was low. The present study also indicated that *vat*, *Cvi*/*Cva*, *iss*, *astA* and *tsh* toxin-encoding genes were present in 18, 19, 47, 29 and 1% of all UPEC isolates.

Our results highlight the higher frequency of genes coding for iron uptake systems, capsular polysaccharide and invasion than toxin encoding genes, indicating the crucial role of these virulence factors in UTI-causing *E. coli*. Therefore, it appears that genes coding for iron uptake systems, capsular polysaccharide and invasion are the most important armaments for adherence to epithelial surfaces and to break host defense systems. These observations confirm the higher prevalence of these virulence genes among our isolates and probably their more significant role in pathogenicity in this geographical region.

Due to differences in social, economic, health, hygiene and environmental conditions between different geographical regions, more studies are needed to detect the virulence factors of UPEC to determine the pattern of pathogenicity in these bacteria. Furthermore, studies are needed to determine the relationship between the expression of virulence factors and antibiotic resistance. These studies will be important in understanding the role of these factors in causing UTIs, which in turn may lead to the development of universal vaccines to prevent such infections.

We concluded that major differences exist in the prevalence of virulence factors among different UPEC isolated from different geographical regions. Detection of these virulence genes may aid in better management of related infections.

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## Footnotes

**Authors' Contribution:**Study design, data collection and data interpretation: Milad Shokohi; study design, data collection, data inter pretation, funds collection, literature review and manuscript preparation: Ahmad Rashki.

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