

Short Communication

Virulence Characteristics and Antibiotic Resistance Patterns among Various Phylogenetic Groups of Uropathogenic *Escherichia coli* Isolates

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SUMMARY: The aim of this study was to determine the resistance patterns of uropathogenic *Escherichia coli* (UPEC) isolates and to investigate the frequency of several virulence genes, including *fimH*, *papA*, *hlyD*, *cnf-1*, *sitA*, and *tsh*, among various phylogenetic groups of UPEC isolates. A total of 85 *E. coli* isolates were recovered from urine samples from outpatients with a clinical diagnosis of uncomplicated urinary tract infections. A molecular approach to examine the antimicrobial resistance patterns was employed using PCR and the disc diffusion method. The detected frequencies of the virulence factor genes determined using PCR were: *fimH* (34.1%), *papA* (9.4%), *hlyD* (21.2%), *cnf-1* (3.5%), *sitA* (15.3%), and *tsh* (27.1%). These results revealed that the isolates were resistant to trimethoprim-sulfamethoxazole (SXT) (74.1%), cefotaxime (CTX) (68.2%), and amoxicillin-clavulanic acid (AMC) (94.1%), and they were relatively less resistant to N (56.5%). According to these results, further investigation is needed to determine exactly whether or not SXT, CTX, and AMC are appropriate antibiotics for the treatment of UPEC infections in southern Iran. Although these results demonstrate that *fimH* is the most frequent virulence gene among UPEC isolates, the high prevalence of isolates that do not encode *fimH* (75.9%) and the relatively low frequency of isolates that carry other virulence genes require further investigation to clarify the role of the other potential virulence factors in the pathogenesis of these isolates.

Escherichia coli strains isolated from the urinary tract are known as uropathogenic *E. coli* (UPEC). UPEC cause 80–90% of community-acquired urinary tract infections (UTI) and 50% of nosocomial UTI (1). The majority of individuals suffering from this type of infection are young women, of which half experience a UTI by age 30. There are some reports that men and children are also exposed to this type of infection (2–4). Annually, UTI is the cause of approximately 8 million physician visits, which costs the United States health care system approximately 1.6 billion dollars (5). Virulence factors involved in UPEC infection include fimbrial and non-fimbrial adhesins, toxins, iron acquisition factors, LPS, and capsules, which facilitate the colonization of bacteria in the urinary tract and invasion into host cells (6).

Various reports have indicated that common antibiotic therapies are not effective in preventing UTIs, since persistent and recurrent infections occur within the bladder (5). Since antibiotic resistance genes are located on mobile genetic elements, their distribution expedites their presence within communities. Thus, a high degree of resistance to various classes of antibiotics has been observed among UPEC strains, resulting in great concern for the treatment of UTIs (7). As the antibiotic re-

sistance patterns of UPEC are different among various countries, there is a need to investigate antibiotic susceptibility profiles for the treatment of UPEC in Iran specifically (8). The aims of this work was to investigate the susceptibility of UPEC isolates to 4 commonly used antibiotics and to determine the prevalence of *E. coli* virulence genes, including toxins (*hlyD* and *cnf1*), adhesins (*papA* and *fimH*), iron transport (*sitA*), and temperature-sensitive hemagglutinin (*tsh*) genes, as well as associations with various phylogenetic groups.

From January 2012 to December 2012, clinically diagnosed patients that were referred to Shiraz hospitals for UTI were selected from various regions of southern Iran. Diagnosis criteria included urge to urinate frequently, sharp pain or burning sensation in the urethra when urine was released, and pyuria. Midstream urine samples were collected in sterile bottles. Patients were selected from both genders and from different ages, with appropriate informed consent. *E. coli* isolates were identified based on standard biochemical methods that have been previously described (9).

The susceptibilities of all *E. coli* isolates to the selected antimicrobials were determined using a disc diffusion method (10). Commercial antimicrobial discs used in this study were trimethoprim-sulfamethoxazole (SXT) (25 µg), cefotaxime (CTX) (30 µg), amoxicillin-clavulanic acid (AMC) (10 µg), and neomycin (30 µg). The *E. coli* strain ATCC 25922 was used as a control strain.

Bacterial DNA extraction was carried out using a boiling method. PCR assays for investigating the presence of the *hlyD*, *cnf-1*, *tsh*, *papA*, *fimH*, and *sitA* genes in *E. coli* isolates were carried out using an MJ Mini thermal cycler (Bio-Rad, Hercules, CA, USA) and

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specific primers that have been used previously (11). PCR mixtures for all reactions contained 2.5 μ L 10 \times PCR buffer, 0.75 μ L dNTP (each at 0.2 mM), 0.2 μ L Taq DNA polymerase (5 U/ μ L), 1 μ L of each primer (20 pmol), 0.75 μ L 50 mM MgCl₂ (CinnaGen, Tehran, Iran), and 3 μ L of template DNA. All amplification procedures were carried out separately except for *papA* and *fimH*, which were amplified using a duplex PCR assay. Amplification reactions for *hlyD*, *cnf-1*, *papA*, and *fimH* were performed as previously described (11). PCR conditions for the detection of *tsh* were as follows: 5 min at 95°C, 30 cycles of 30 sec at 94°C, 45 sec at 55°C, 45 sec at 72°C, and a final extension of 10 min at 72°C. PCR products were transferred and electrophoresed on a 2 % agarose gel containing ethidium bromide (CinnaGen) and visualized using a UV trans-illuminator.

In order to identify significant relationships between phylogenetic groups of *E. coli* isolates, the presence of virulence genes, and correlations among *E. coli* virulence genes, Fisher's exact tests (SPSS version 19.0; SPSS Inc., Chicago, IL, USA) were performed. *P*-values less than 0.05 were considered statistically significant.

A total of 85 UPEC isolates were identified from patients using conventional biochemical identification. According to a previously published report, 56 (65.9%) of these isolates belonged to phylogenetic group A, 15 (17.6%) belonged to phylogenetic group B2, and 14 (16.5%) of the isolates were found to belong to group D (9).

According to the present study, the prevalence of resistant isolates against AMC, SXT, CTX, and neomycin was 94.1%, 74.1%, 68.2%, and 56.5%, respectively. In addition, 36.4% of the UPEC isolates exhibited multidrug-resistance properties. The incidence of antibiotic resistance within the phylogenetic groups is summarized in Table 1. In contrast with WHO recommendations for UTI treatment with SXT, many studies have proposed SXT as an inappropriate antibiotic for the treatment of UTI (12–15). Abass et al. have indicated that UPEC strains not only display high resistance

to SXT (72%) but also showed 100% resistance to AMC, which is in accordance with the present results (13). In India, Murugan et al. have demonstrated a significant resistance level (74%) of *E. coli* isolates against cefotaxime in patients with UTI, which is comparable to our findings (16). Ramos et al. have shown that there is higher rate of resistance of UPEC isolates from Iran and Vietnam to AMC, compared with 3 other countries. In addition, this report described a high prevalence (44%) of multidrug-resistant UPEC isolates from Iran, which is similar to our observations (14). In one study, the frequency of UPEC strains resistant to neomycin (37.8%) was less than that of the other studied antibiotics (17). According to the present study, due to high-level resistance of isolates against AMC, SXT, and CTX, we conclude that these antibiotics are relatively ineffective for UTI treatment in Southern Iran. However, neomycin could be considered as an efficient treatment for UTIs.

The presence of the above-mentioned virulence genes in the 85 UPEC isolates was determined using PCR assays. It is noteworthy to mention that 35 (41.1%) of these strains did not encode any of these virulence genes. The prevalence of the virulence genes among the phylogenetic groups of the isolates is shown in Fig. 1. Of the UPEC adhesins, FimH at the type 1 fimbria tip

Table 1. Distribution of antibiotics resistance genes among UPEC strains

| Antimicrobial agent | N (%) of antibiotic resistance isolate in phylogenetic group | | | |
|---------------------|--|-----------|-----------|---------------------|
| | Type A | Type B2 | Type D | Resistance isolates |
| N | 29 (34.1) | 11 (12.9) | 8 (9.4) | 48 (56.5) |
| SXT | 41 (48.2) | 12 (14.1) | 10 (11.8) | 63 (74.1) |
| CTX | 39 (45.9) | 10 (11.8) | 9 (10.6) | 58 (68.2) |
| AMC | 53 (62.4) | 15 (17.6) | 12 (14.1) | 80 (94.1) |

N, neomycin; SXT, trimethoprim-sulfamethoxazole; CTX, cefotaxime; AMC, amoxicillin-clavulanic acid.

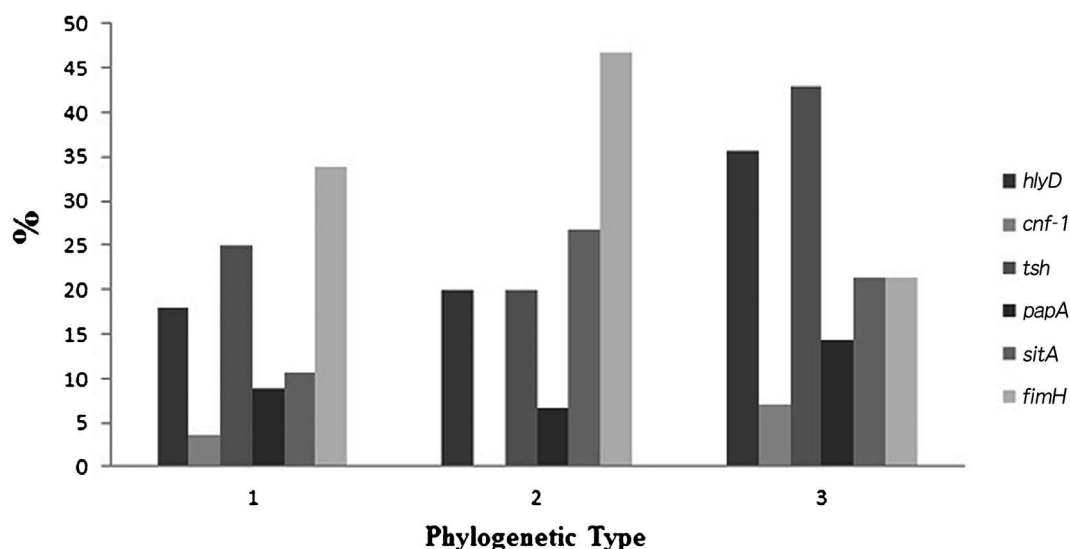


Fig. 1. Distribution of virulence genes detected in 3 phylogenetic groups of UPEC. 1, 2, and 3 represent phylogenetic groups A, B2, and D respectively.

has a crucial role in UPEC colonization in the bladder, which is required for the initiation of UTI (4). According to the present observations, *fimH* had the highest frequency (34.1%) among the virulence genes, while *cnf-1* had the lowest (3.5%). Several studies have shown that *fimH* is the most prevalent among these virulence factors, and it has been detected in 67%, 86%, and 87% of UPEC isolates in 3 separate studies performed in Iran (18–21). Although the results presented here confirm that *fimH* is the most frequent virulence gene among UPEC isolates, the frequency of isolates harboring *fimH* dramatically differed from the majority of the above-mentioned reports. These divergent findings might result from differences in UPEC isolates derived from various populations living in different geographical regions. Moreover, our findings bring up a challenging question about the mechanism of pathogenesis in 75.9% of our UPEC isolates that did not harbor *fimH*. It appears that further investigation is essential in order to clarify the pathogenesis mechanism and detect other potential virulence factors in UPEC isolates that do not harbor *fimH*. The major subunit of p-fimbriae is encoded by the *papA* gene in UPEC. This fimbria is involved in the colonization of UPEC in the urinary tract and in the development of pyelonephritis (6,22). Our results indicate that the *papA* gene is present in 9.4% of the UPEC isolates. Interestingly, all of the isolates encoding *papA* carried the *fimH* gene, and our statistical analysis revealed a significant association between the presence of the *papA* and *fimH* genes ($P < 0.05$), which warrants further investigation. However, the results of the present study are not in accordance with previous studies that have determined the *pap* frequency in UPEC isolates in Iran (30% and 30.2%), Tunisia (41%), and China (54%) (12,19,21,22). In addition, statistical analysis revealed significant relationships between the presence of *papA* with that of the *tsh* and *sitA* genes ($P < 0.05$). The *hlyD* gene participates in the transport of hemolysin; therefore, it is involved in UPEC-mediated hemolysis (6). Cytotoxic necrotizing factor-1, a Rho GTPase encoded by *cnf-1*, is involved in invasion into the host cells (4). In this report, the results indicated that 21.2% and 3.5% of UPEC isolates harbored the *hlyD* and *cnf-1* genes, respectively, and none of the isolates carried both of these genes. Interestingly, there are several contradictory publications pertaining to the presence of these 2 toxin related genes. Both toxin genes, *hly* and *cnf-1*, were detected by Karimian et al. in 50.4% of UPEC isolates in Tehran, Iran (20). However, Tarchouna et al. reported the prevalence of *hly* and *cnf* in 19% and 3% of isolates, respectively, which are comparable to the present findings (22). Statistical analysis also indicated significant correlation between the presence of *hlyD* with *papA*, *sitA*, and *tsh* ($P < 0.05$). In addition, likely due to the low prevalence, there were no statistically significant correlations between *cnf-1* and the other virulence genes. The *sitA* gene is one of the iron acquisition-related genes in *E. coli* and encodes a component of the ABC iron transport system (23). Our findings indicated that *sitA* was present in 15.3% of the UPEC isolates. To the best of our knowledge, there are no published data pertaining to the presence of *sitA* in UPEC isolates from Iran. Previously, this gene was detected in approximately 77% of UPEC isolates causing

recurrent UTI in China and in 85.5% of UPEC isolates in the United States (15,24). In the study performed by Luo et al. (*sitA* = 77%), UPEC phylogenetic group D was the most prevalent, followed by groups B2 and then A (24). However, our findings indicate that the frequency of phylogenetic group A was more than twice that of the others. The divergent findings on the prevalence of *sitA* in UPEC obtained in our study and the previously mentioned study might be a result of this significant difference in the phylogenetic groups. Temperature-sensitive hemagglutinin, encoded by *tsh*, is an autotransporter protein that is involved in UPEC pathogenesis (25). This gene was found in 27.1% of our studied UPEC strains. The presence of this gene was formerly reported in 39.5% of UPEC isolates (11). However, a previously published report in Iran determined there were no UPEC strains containing *tsh* in its study population (18). Identifying *tsh* in 27.1% of the samples and determining a significant correlation between its presence and that of *papA* and *hlyD* are hallmarks of our study. Overall, these contradictions among the data collected in different studies might stem from the fact that the specimens in these various studies were obtained from different geographical regions of the world, including studies performed using samples solely collected from Iran from cities that are hundreds to thousands of kilometers away from each other and from different populations varying in lifestyle. In recent years, multidrug resistance among UPEC strains has increased, and these strains have become a public health concern in several countries (4). Therefore, research on developing new therapies for UPEC-derived UTI is certainly a priority for the future. One of these new therapies has been developed by Totsika et al. who proposed an inhibitor of FimH as a potential therapeutic for the treatment of multidrug-resistant UPEC infections (15).

The current investigation has some limitations, which will be eliminated in further studies as much as possible. One is the relatively small number of samples that were investigated. Moreover, the antibiotic resistance patterns of the studied UPEC isolates would be more reliable if the presence of the antibiotic resistance genes were studied using molecular approaches such as PCR. Nevertheless, this study has several important outcomes. First, the present study determined that high resistance to AMC, SXT, and CTX is common among the investigated UPEC isolates. In addition, a low rate of resistance to neomycin has been shown among these isolates. However, due to the relatively small size of our study population, we are not able to make direct conclusions pertaining to these findings. Therefore, it appears that more in-depth, large-scale studies are required to investigate the antibiotic resistance patterns among UPEC isolates in southern Iran in order to confirm or reject the current findings. Another important outcome of this study is the detection of *fimH* among the UPEC isolates as the most frequent virulence gene. This confirms the crucial role of *fimH* in UPEC pathogenesis and necessitates further studies on *fimH* inhibitors as potential backup therapeutics for UPEC-associated UTI. In fact, the obtained data on the frequency of virulence genes can be useful in designing new targets for UPEC antibiotic-independent therapies. However, the most remarkable findings were detecting the high

prevalence of isolates that do not harbor *fimH* (75.9%) and the relatively low frequency of isolates carrying the other virulence genes. These significant findings suggest that further investigation should be conducted in order to clarify the role of other potential virulence factors in the pathogenesis of these isolates, especially for *tsh*, which was detected with a significantly higher frequency in this study compared with others in Iran.

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Conflict of interest None to declare.

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