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Original Article

Determination of the Frequency of Adhesion Virulence Factors in Uropathogenic *E. coli* (UPEC) Strains Isolated from Hospitalized Patients in Babol

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Background and Aims: Uropathogenic *E.coli* is one of the most known causes of urinary tract infections, which may lead to a high rate of morbidity and mortality in high risk patients. In this regard, the virulence factors such as bacterial adhesion molecules have a critical role. The current study was intended to determine the molecular properties of adhesion genes in Uropathogenic *E.coli* strains isolated from hospitalized patients in Babol, north of Iran.

Materials and Methods: During a nine-months of study, 90 Uropathogenic *E.coli* strains were confirmed by differential biochemical and microbiological standard tests, antimicrobial susceptibility test, and molecular polymerase chain reaction assay were obtained for evaluating the frequency of adhesion genes.

Results: According to the results, the highest rate of resistance and susceptibility were belonged to penicillin and imipenem respectively. On the other hand, the percentage of *fimH* gene among other virulence genes was reported to be 66%.

Conclusions: The present study showed that a high level of uropathogenic *E.coli* isolates which harbored the adhesion factors may lead to distribution of multiple antimicrobial resistance strains.

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Introduction

Urinary tract infection is one of the most common bacterial infections that occurs in all age groups, in which *Escherichia coli (E. coli)* is known as a significant etiologic factor in 50-80 percent of cases [1]. The severity of the urinary tract infections of this bacterium is due to the presence of a wide range of virulence factors. Presently, the general hypothesis is that pathogenic strains are derived from non-pathogenic strains due to horizontal gene transfer process. These strains contain various virulence factors (VFs) such as adhesions, toxins, siderophores and the like contributing. which contribute to the development of the infection procedure and each subsequently overcome the host immune system [2]. However, the various adhesion genes such as *fimH*, *papC*, i*ucC*, *sfa/foc* are involved in virulence, colonization and invasion [3]. Isolates that harbor these genes can lead to the emergence of multi drug resistant (MDR) strains [4]. The global investigations indicate that the antibiotic resistance increases among urinary tract infections due to the variety of virulence factors, albeit usage of antimicrobial agents, microbial properties and therapeutic strategies are critical in this regard. The aim of this study was to recognize the frequency of various adhesion genes and also the antimicrobial susceptibility profile of collected *E. coli* strains from urinary tract samples.

Materials and Methods

Clinical samples and laboratory identification

In this cross-sectional study a total of 90 non-duplicative clinical isolates of Uropathogenic *E.coli* were collected over nine months from urinary tract infection (UTI) samples (defined as the presence of a positive urine culture $\geq 10^5$ CFU/ml and pyuria) of patients admitted to Ayatollah Rouhani Hospital in Babol, north of Iran. The study was approved by the Ethics Committee of Babol University of Medical Sciences, Babol, Iran (No: MIBABOL.REC.1395.246). The isolates were confirmed by conventional biochemical, microbiological and API 20E test system (BioMerieux Inc, France). All strains were stored in liquid broth (Merck, Germany) containing 20% glycerol at -20°C for molecular usage.

Antibiotic susceptibility test

In accordance with the Clinical and Laboratory Standards Institute (CLSI document M100-S16) guidelines, the antimicrobial susceptibility profile was conducted on the Mueller-Hinton agar plates (Merck, Germany) using the standard disk diffusion method for following antimicrobial agents: Penicillin (10 µg), Nalidixic acid (30 µg), Cefixime (5 µg), Gentamicin (10 µg), Nitrofurantoin (300 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Co-trimoxazole (25 µg), Cefotaxime (30 µg), Ceftazidime (30 µg) (Rosco, Denmark).

Polymerase chain reaction (PCR) method

DNA genomic was extracted by genomic DNA purification kit (Yekta-Tajhiz, Iran) as a recommendation protocol. Specific primer sequences used in this study are listed as below: *sfa/foc* (F:GGAGGAGTAATTACAAACCTGGCA,

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R: GAGAACTGCCCGGGTGCATACTCT) (410 bp), *PapC* (F: GACGGCACTGCTGCAGGGTGTGGCG, R: ATATCCTTTCTGCAGGGATGCAATA) (328 bp), *fimH* (F: TGCAGAACGGATCCGTGG, R: GCAGTCACCTGCCCTCCGGTA) (508 bp), and *iucC* (F: AAACCTGGCTTACGCAACTGT, R: ACCCGTCTGCAAATCATGGAT) (269 bp) (Bioneer, Korea) [3]. The total volume of the PCR reaction mixture was 25 µl, containing 1 µl of extracted template DNA, 2 µl of 10× PCR buffer, 0.6 µl $MgCl_2$ (50 mM), 0.6 µl dNTPs (10 mM), 0.5 µl of each primers, 0.5 µl of Taq DNA polymerase (5 U/µl) (Amplicon Co., Denmark) and 19.3 µl double distilled water. Amplification was carried out in a Techne TC-512 thermocycler (Eppendorf, Hamburg, Germany) as follows: initial denaturation at 95°C for 5 min, 30 cycles of denaturation for 30s at 94°C, annealing for 30s at 57°C, extension for 60s at 72°C, and a final extension for 5 min. at 72°C. Finally 5 µl of each PCR product was electrophoresed at 80V for 1 hour in a 1% agarose gel, stained and visualized with ultraviolet illumination system (Bio-Rad, Hercules, USA).

Results

During the period of study, 90 UTI *E. coli* strains were obtained from different parts of Ayatollah Rouhani Hospital, (Babol, Iran) out of which 62.6% were above 50 years old with an average age of 55. Also, the patients' gender comprised of 40% male and 60% female. The most percentage of the samples was isolated from infection disease (33.3%) and hematology-oncology wards (19%). The antimicrobial resistance pattern illustrated that penicillin with (100%) and imipenem by (0%) had the highest and lowest ratio. All examined antimicrobial agents are demonstrated in Fig 1. On the other hand, 43.3% of isolates were distinguished as MDR strains. By PCR molecular test the *fimH* with 66% had the highest rate among other genes while, the other genes were followed as: *papC* (46.7%), *iucC* (44.12%) and *sfa/foc* (39.4%). The PCR amplification of *fimH*, *papC*, *iucC*, and *sfa/foc* genes is presented in Figs 2-5.

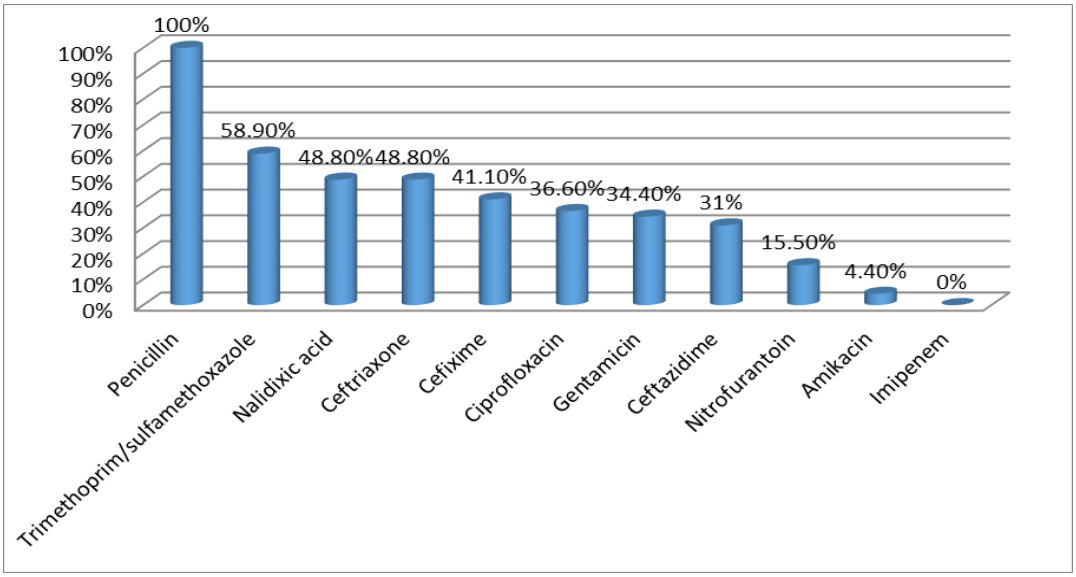

Fig. 1. Antibiotic resistance profile of collected *E. coli* strains

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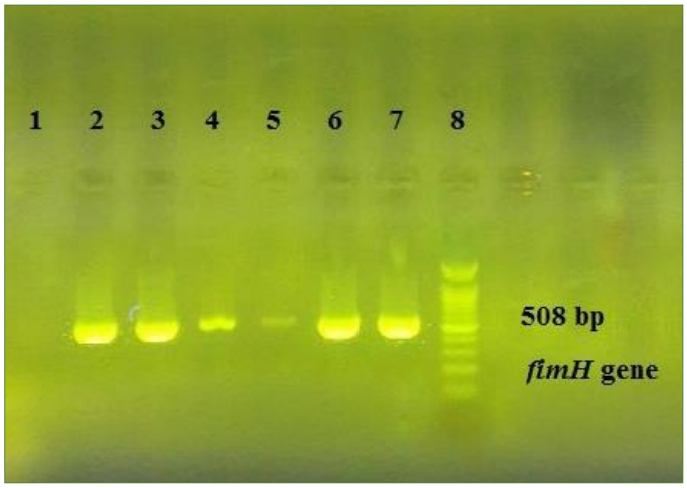

Fig. 2. Agarose gel electrophoresis analysis for the *FimH* gene Line 1: Negative control, Lines 2-6 positive strains with *fimH* gene, Line 7: Positive control, Line 8: 100 bp *DNA* size marker

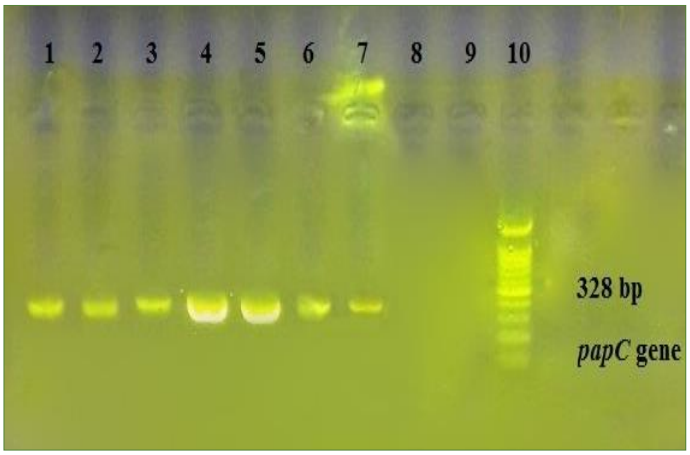

Fig. 3. Agarose gel electrophoresis analysis for the *papC* gene Lines 1-6: Positive strains with *papC* gene, Line 7: Positive control, Line 8: Negative control, Line 9: Negative strain, Line 10: 100 bp DNA size marker

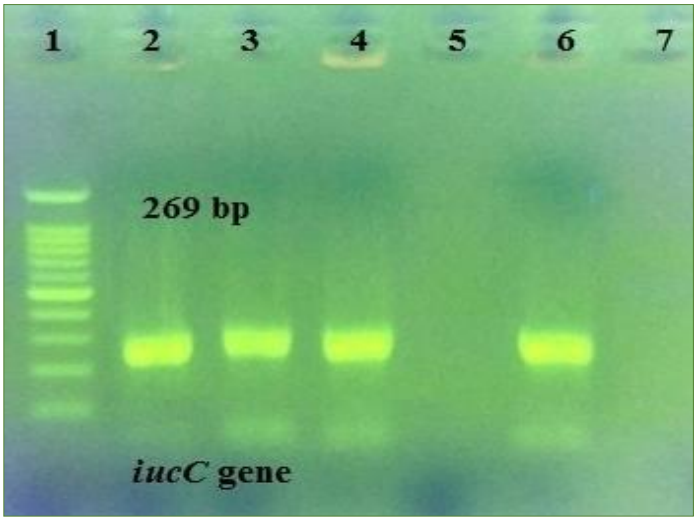

Fig. 4. Agarose gel electrophoresis analysis for the *iucC* genes Line 1: 100 bp DNA size marker, Line 2: Positive control, Line 3, 4 and 6: Positive strains with *iucC* gene, Line 5: Negative control

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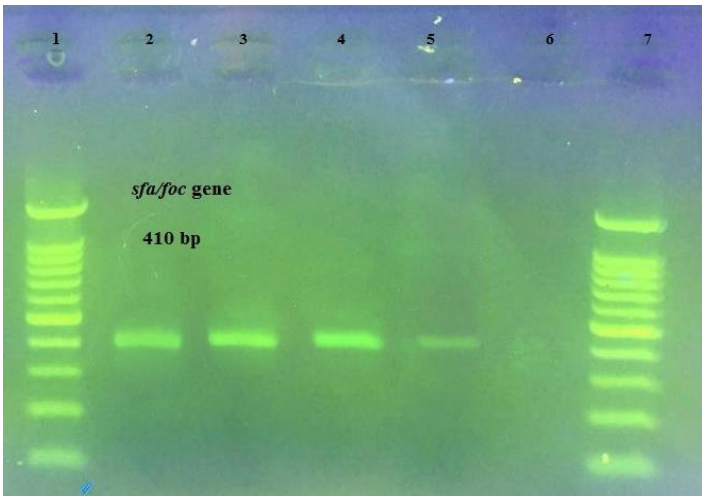

Fig. 5. Agarose gel electrophoresis analysis for the *Sfa/foc* gene Line 1 and 7:100 bp DNA size markers, Line 2: Positive control, Lines 3-5: Positive strains with *sfa/foc* gene, Line 6: Negative control.

Discussion

According to some studies, the age of the patients may be an important risk factor in UTI, although in our results, the patients with more than 50 years old were sensitive to Uropathogenic *E.coli* infections [5, 6]. The antimicrobial susceptibility results were indicated that among tested antimicrobial agents, the imipenem (0%), amikacin (4.4%) and nitrofurantoin (15.5%) are the most effective antibiotics against Uropathogenic *E.coli* strains in the present study. Additionally, 43.3% of isolates were recognized as MDR strains. The prevalence of *fimH* gene (66%) may prove the mentioned issue in the present study for the reason that it can contain the attachment of *E.coli* to mucosal surfaces and initiate the UTI infection. Also, the similar studies have reported similar results around the high frequency of *fimH* gene in *E.coli* strains [7-9]. Moreover, the *papC* gene with 46.7%, which is responsible for the bacterial fimbriae function, has a significant role in attachment to eukaryotic cells [10]. Moreover, the *iucC* gene, which is responsible for the synthesis of the hydroxamate siderophore, and the other adhesion factor, *sfa/foc* gene, were determined 44.12% and 39.4% respectively. In López-Banda et al. study the prevalence of *fimH* and *papC* genes were 86.1% and 62% respectively [11]. However, in Tiba et al.'s study, the percentage of adhesion factors in patients with cystitis was reported as follows: *fimH* (97.5%), *papC* (32.7%), and *iucC* (25.9%) [12]. In the same study in Romania, the prevalence of *fimH*, *sfa/foc* and *papC* were 86%, 23% and 36%, respectively [13]. Chakraborty and colleagues (2015)

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demonstrated that the percentage of *fimH* and *papC* genes in 100 studied isolates amounted to 76% and 44%, respectively [14]. One of the differences between reported results may be related to the frequency of virulence factors of various strains and distribution of uropathogenic *E.coli* strains in various regions [15-18]. Moreover, in a current study by statistical analysis, there was a relationship between resistance to ciprofloxacin, gentamicin with *papC*, co-trimoxazole and *fimH*, *iucC* positive strains and resistance to cefixime (p-value <0.05). Furthermore, 30% of all strains harbored two genes, 20% of them harbored three genes and 11.1% had 4 positive genes simultaneously.

Conclusion

Uropathogenic *E.coli* is one of the main causes of UTI in different patients, hence the evaluation of the bacterial adhesion factors and the therapeutic approaches are the main aspects in UTI treatment. Due to an increase in the resistance genes in different conducted studies the dissemination of MDR strains is not unexpected.

Conflict of Interest

None to declare.

Acknowledgement

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