Short Communication

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The Frequency of pap and sfa Genes Among Escherichia Coli Strains Isolated from Hospitalized Patients of Rouhani Hospital in Babol, Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Escherichia coli urinary tract infections are known as one of the most important nosocomial infections. Adhesion genes such as *sfa* and *pap* which are important in bacterial attachment and colonization in epithelial cells have a significant role in bacterial pathogenecity. The aim of this study was to investigate the frequency of mentioned genes among *E. coli* strains isolated from hospitalized patients of Rouhani hospital in Babol city.

METHODS: A total of 70 E. coli strains were isolated from urinary specimens of Rouhani hospitalized patients and then identified and confirmed with differential tests by using MacConkey agar, TSI, Oxidase test and etc. Thereafter, antimicrobial pattern were carried out by disk diffusion method. Finally the bacterial genomic DNA was extracted and the frequency of genes was determined by molecular method.

FINDINGS: In this study resistance to Erythromycin (65.7%) and Trimethoprim-sulfamethoxazole (57%) had the highest resistance ratio. On the other hand the frequency of *sfa* and *pap* genes was 60% and 27% respectively.

CONCLUSION: The results of this study showed the sfa and pap genes have a high prevalence and antibiotic resistance also needs special attention.

KEY WORDS: *Escherichia coli, Urinary Tract Infections, Antibiotic Resistance*.

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Introduction

Bacterial infections are one of the most important and common causes of Urinary tract infections, which can lead to excessive expenses in the health sector (1). In developing countries, about 150 million people get infected with urinary tract infections annually (2). According to studies, the incidence rate in female is higher than males (3). Among the bacterial agents, Escherichia coli are the most important factor in causing this type of infection. This bacterium is the dominant microorganism of the intestinal flora, which can lead to extra-intestinal infections such as pyelonephritis, bacteremia, osteomyelitis, pneumonia, cystitis, and etc (4). On the other hand, Escherichia coli are known as a factor of about 35% of hospital acquired infections (5).

Escherichia coli can be colonized by means of adhesion factors under conditions such as immune deficiency or underlying diseases and ultimately lead to the bladder and kidneys, leading to cystitis and urinary tract infection (4). Laboratory and clinical evidence suggests that strains that have the ability to infect have different virulence factors that can play an important role in attachment of bacteria to uroepithelium and colonization and pathogenicity. The bacterial attachment to the uroepithelium allows bacteria to resist bladder drainage and activate the signaling pathway in the host.

Hence, bacterial attachment to uroepithelium is an important step for the onset and development of urinary tract infection (3). This attachment occurs through a bacterial adhesins named Fimbria P, coded by the pap gene or pyelonephritis associated pili (3). Fimbria P plays a role in the bacterial colonization in the upper urinary tract, attachment to the endothelium of the renal arteries and eventually the development of pyelonephritis (6).

Another important adhesin factor for this issue is Fimbria S, coded by sfa or S fimbria adhesin. The above genes are mannose-resistant adhesins and are located on a region of the chromosome called pathogenic islet (3-5). According to the studies, there is a significant relationship between pap and sfa genes and pyelonephritis, meningitis and septicemia (7). Among these adhesional factors, other pathogenic factors, such as aerobactin, colisin and hemolysin, are also attributed to E. coli (8). Therefore, according to the above, rapid diagnosis of virulence factors using PCR technique and antibiotic resistance testing is necessary to determine the therapeutic protocol. The study of these genes has been evaluated among E. coli strains in various geographic regions of the world as well as in Iran. However, since the study about this issue has not been done in Babol city, the aim of this study was to determine the frequency of pap gene and sfa in Escherichia coli strains isolated from patients admitted to Ayatollah Rouhani Hospital in Babol during a six-month period.

Methods

This cross-sectional study was approved by the Ethics Committee of Babol University of Medical Sciences with the code of 247.1395. MUBABOL.REC and was performed on urine specimens isolated from patients admitted in Rouhani Hospital in Babol. In a six-month period, referring to Ayatullah Rouhani Hospital's lab, 70 strains of Escherichia coli from urine specimen from patients diagnosed with urinary tract infection confirmed by a physician, were isolated and sent to the Department of Microbiology of Babol University of Medical Sciences.

In the direct urine analysis, the samples were examined for the presence of WBC, epithelial cells, bacteria and mucus. Using standard microbiological tests such as culture on the MacConkey agar, gram staining, differential tests such as endol tests, Simon Citrate, SIM, TSI, MR / VP, etc, E. coli strains were confirmed. At the end, strains were stored in a BHI broth medium containing 15% glycerol at -20 °C for subsequent experiments.

Antibiogram test: Antibiotic resistance test was done by Kirby Bauer method using antibiotic discs such as erythromycin (15µg), trimethoprim-sulfamethoxazole (1/25 + 1/75 + 23µg), ciprofloxacin (5µg), gentamicin (10µg), Tobramycin (10µg), levofloxacin (5µg) and imipenem (10µg) according to CLSI (2016) standards.

DNA extraction: DNA extraction was done using the ROCHE company kit based on the manufacturer's protocol.

PCR Test: To perform this test, specific primers tested using Blast software and special programs were used (Table 1). The final volume for each reaction was considered 25 µl. The PCR program included initial denaturation at 94 ° C for 4 minutes and 35 cycles, each cycle including three stages of denaturation at 94°C for 45 seconds, annealing at 63 ° C for 45 seconds, amplification the primer in 72 ° C for one minute and 30 seconds, and final amplification at 72 °

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C for 5 minutes. After completion of the reaction, the PCR product was examined using 1% agarose gel by transluminator device.

Table1. Specific primers for investigating pap and sfa genes.

Gene	Primers	size (bp)
pap	GCA ACA GCA ACG CTG GTT GCA TCA T AGA GAG AGC CAC TCT TAT ACG GAC A	336
sfa	CGG AGG AGT AAT TAC AAA CCT GGC A CTC CGG AGA ACT GGG TGC ATC TTA C	410

Results

Of the 70 strains collected from Escherichia coli, 53% and 47% were belonged to female and male, respectively. Based on the antibiotic resistance pattern, 65.7% of the strains were resistant to erythromycin and 57% to trimethoprim/sulfamethoxazole. On the other hand, 41.5% were resistant to ciprofloxacin, 32.8% to gentamicin, 27.2% to tobramycin, 12.8% to levofloxacin, and 4.3% to Imipnem. Among the studied strains, the frequency of strains with multiple resistances was 15.7%. In examining the frequency of the studied genes, 27% of strains had pap gene and 60% had sfa gene. Figures 1 and 2 illustrate the PCR of these genes.

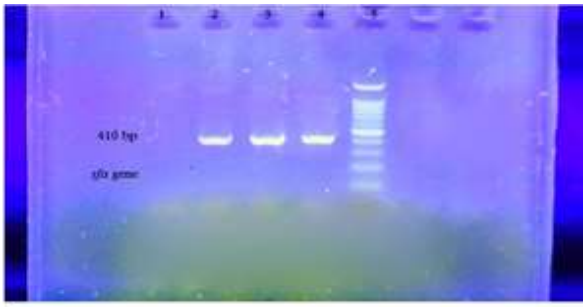

Figure 1. Line 1 Negative Control, line 2 and 3 strains of sfa gene, line 4 positive control and line 5 100 bp DNA Ladder.

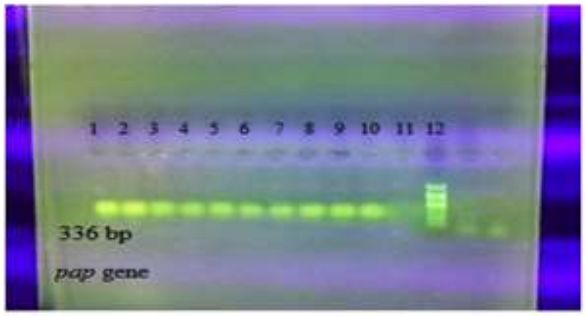

Figure2. Line 1, 2, 3, 4, 5, 6, 7, 8, 9 strains of pap gene, line 10 positive control, line 11 negative control and line 12 100 bp DNA Ladder.

Discussion

In the present study, the frequency of sfa and pap gene was 60% and 27%, respectively. Escherichia coli bacteria are one of the most important causes of urinary tract infection (1). The presence of fimbria in pathogens of E. Coli is an important factor in the pathogenicity of the bacterium and, along with other pathogenic factors, can play a significant role in pathogenesis. The presence of factors involved in adhesion plays a very important role in the binding of bacteria to the urinary tract and its colonization. In the study of Tarchouna et al. 90 strains were examined, in which 41% of the strains had sfa gene and 34% had pap gene (9).

In a study by Karimian et al., studying of 123 strains of E. coli collected from urinary tract infections the sfa gene was reported 14/6% and the Pap gene was reported 27% (10). In the study of Rahdar et al., the frequency of sfa and pap gene was reported as 81% (7). Also, in the study of Asadi et al., the frequency of both genes was 53.3% (11). In the study of Bahalo et al., the genes of sfa and pap were found to be 30% and 40% respectively (12). On the other hand, in the reports of Mohajeri et al. (20.5%), 42 strains had pap gene and (21.5%) 44 had the sfa gene (13). Also in Shetty et al. the frequency of pap gene was 61.87% and the sfa gene was 39.3 %(14).

The variation in the abundance of these genes in studies may be due to strain dispersion in different geographical regions. Since the first step in the pathogenicity of E. coli is its attachment to the wall of the uroepithelium, so the fimbria and adhesion factors play a key role. On the other hand, pyelonephritis and complicated urinary tract infections have a significant relationship with the presence of genes encoding adhesion factors, so their rapid diagnosis can be helpful in treating patients.

Considering the importance of this issue, the abundance of these genes was studied in Rouhani Hospital of Babol, with similar results reported in other parts of the country. On the other hand, the presence of several antibiotic resistance strains was also evaluated in this study, which was partially quantitative, but resistance to imipenem antibiotic can lead to health problems in the near future therefore,using the therapeutic strategies can prevent the emergence of these strains.

Conflict of Interest: No conflicts of interest.

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