Original Article

Genotyping of Virulence Factors of Uropathogenic *Escherichia coli* by PCR

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Abstract

Background: *Escherichia coli* is the most causative agent of urinary tract infections (UTIs). Apart from all human infectious diseases, UTI have a high prevalence and in most cases, *Escherichia coli* is a dominance bacterium which can cause pyelonephritis and cystitis. The aim of the study was to determine the occurrence of some virulence genes expressing fimbriae, production of hemolysin and aerobactin among a hundred *Escherichia coli* isolates obtained from in-and outpatients of Karaj Shahid Rajaii hospital, showing clinical and laboratory signs of UTI.

Materials and Methods: In this investigation we isolated *Escherichia coli* strains from urine samples of patients with UTI during the period of July to December 2012 and studied them for the presence of the virulence genes by PCR.

Results and Conclusion: The most abundant virulence factor in this study was *fimH*. The prevalence of the virulence factors for fimbriae type 1 (*fimH* gene), pyelonephritis associated pili (*pap* gene), S-family adhesions (*sfa* gene), hemolysin (*hly* gene) and aerobactin (*aer* gene), was 73%, 46%, 32%, 47%, 57%, respectively.

Keywords: *Escherichia coli*, Urinary tract infection, Virulence factors, PCR

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Introduction

Urinary tract infection is one of the most prevalent of human infections that establishes by *Escherichia coli*. At least 20% of women experience an acute symptomatic urinary tract infection during their lives. The severity of the infection depends on virulence of infecting bacteria and susceptibility of their hosts[1]. Urinary infections often occur in patients with anatomically and functionally normal urinary tract, and involve spontaneous ascent of bacteria from the urethra to bladder and in a few patients to kidney. Adhesion of *Escherichia coli* to uroepithelium may protect the bacteria from urinary lavage, increasing their ability to reproduce and invade renal tissue[1]. The severity of UTI produced by *Escherichia coli* is expanded by the existence of a wide range of virulence factors. The most accepted theory today is that uropathogenic *Escherichia coli* (UPEC) germinated from non-pathogenic strains by gaining new virulence factors via accessory DNA horizontal transfer often organized into clusters (pathogenicity islands) located at chromosomal locus[2]. The dominant of these virulence factors are sticky agents that help to

colonization of bacteria in sites such as urethra or toxin that effects on the host. The UPEC possess adherence factors called pili or fimbriae, which allow them to successfully initiate infections. Specific adhesion is mediated by bacterial proteins termed adhesions which may or may not be associated with fimbriae. *Pap* (pyelonephritis associated pili), *sfa* (S fimbrial adhesin) operons are most commonly found encoding P, S fimbriae respectively[3]. Besides bacterial adherence, several virulence factors may contribute to the pathogenicity of UPEC, including the production of hemolysin and aerobactin[4].

In the present study, we analysed urinary tract *Escherichia coli* isolates to search for possible evidence of a correlation between biological characteristics that could represent pathogenicity traits of these strains.

Methods

A total of 100 strains of *Escherichia coli* isolated from 100 in-and outpatients of Karaj Shahid Rajaii hospital during the period of July to December 2012 were collected and the samples distributed as: 1 subject aged between 0 and 9 years, 3 aged between 10 and 19 years, 15 aged between 20 and 29 years, 13 aged between 30 and 39 years, 14 aged between 40 and 49 years, 21 aged between 50 and 59 years, and 33 aged >59 years, were analysed. Thirty-one of the subjects were hospitalized (inpatients) and sixty-nine were outpatients. UTI diagnosis was established by the hospital medical staff based on clinical symptoms and laboratory methods. The laboratory criterion for acute *Escherichia coli* UTI was the presence of a positive culture with at least 10^5 CFU of *Escherichia coli* per mL of clean-voided urine. *Escherichia coli* was identified with the use of standard methods. The strains were stored in TSB/glycerol at -70°C for further analysis.

DNA Extraction

Escherichia coli isolates were subcultured overnight (18 h) in TSB at 37°C. DNA was extracted by using DNA Extraction Kit (Bionner Company, South Korea) according to manufacturer′s instruction.

PCR

Specific primers were used to amplify sequences of the *fimH*, *pap*, *sfa*, *hly* and *aer* genes as indicated in table 1. Primer sequences, predicted sizes of the amplified products, and specific conditions were described by Monique Ribeiro TIBA et al[3].

The PCR assay was carried out in a total volume of 25 µL of mixture containing 12.5 µL mastermix (Sinaclon Co.), 1 µL of each of the virulence gene-specific primers, 3 µL of template DNA and 7.5 µL of WFI (water for injection). The amplification conditions included three steps: heating at 94°C for 3 min**;** 35 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 30 sec (in related to *fimH* gene 58°C for 30 sec), and extension at 72°C for 3 min**;** and the final extension at 72°C for 7 min[6].

The PCR products were analysed by 2% agarose gel electrophoresis, after which the gel was stained with Ethidium Bromide and photographed[7].

Results

The occurrence of virulence factors in the present study ranged from 32.0% for *sfa* to 73.0% for *fimH*, and for *pap*, *hly*, *aer* was 46%, 47%, 57%, respectively. Among the adhesins, the fimbriae type 1 and P fimbriae were the most prevalent (73, 46 strains) (Table 2). The PCR products were sequenced for identification and authenticity was recovered.

The frequency of uropathogenic *Escherichia coli* virulence factors isolated from patients of Karaj Shahid Rajaii Hospital was shown in table 3.

Discussion

Escherichia coli is the most causative agent of urinary tract infections in both ambulatory and hospitalized

Figure 4. M (DNA ladder 100 bp, Fermentase co.), 3 and 4 positive for *pap* (336 bp), 6, 7 and 8 positive for *aer* (602 bp), 10 and 11 positive for *hly* (1177 bp).

Table 1: Primers used for detection of virulence genes in UPEC strains.

Primer	Identified Gene	Gene Sequence	Product size bp
PAP3 PAP4	*papE/F*	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	336
fim1 fim2	*fimH*	GAGAAGAGGTTTGATTTAACTTATTG AGAGCCGCTGTAGAACTGAGG	508
sfa1 sfa2	*sfaD/E*	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410
aer1 aer2	*aer*	TACCGGATTGTCATATGCAGACCGT AATATCTTCCTCCAGTCCGGAGAAG	602
hly1 hly2	*hlyA*	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177

Table 2: The pattern of uropathogenic *Escherichia coli* isolated from patients of Karaj Shahid Rajaii Hospital based on virulence factors.

Profile	*fim H*	*pap*	*sfa*	*hly*	*aer*	No. of strains
Ec1	−	−	−	−	−	10
Ec2	+	−	−	−	−	23
Ec3	+	+	+	+	+	11
Ec4	+	+	−	+	+	11
Ec5	+	+	−	−	+	10
Ec6	+	−	+	+	+	4
Ec7	+	+	+	−	+	3
Ec8	+	−	−	+	+	3
Ec9	−	−	−	+	+	3
Ec10	−	−	+	+	+	3
Ec11	+	−	+	−	+	2
Ec12	+	−	+	+	−	2
Ec13	−	+	−	+	+	2
Ec14	−	−	+	−	+	2
Ec15	+	+	+	+	−	1
Ec16	+	+	−	+	−	1
Ec17	−	+	−	−	+	1
Ec18	−	+	+	+	−	1
Ec19	−	+	+	+	+	1
Ec20	−	−	+	−	−	1

patients. These infections can diverge from cystitis to a pyelonephritis. The degree of severity depends on the virulence of the responsible strains and on the susceptibility of the host. A better cognition of the virulence characteristics of the microorganism causing infection will permit the clinician to anticipate the evolution of infection in the host[8]. Many virulence determinants contribute to the pathogenicity of *Escherichia coli* in UTI. They are the products of different genes which can be detected by PCR[5]. However there is always the possibility of mutation at the level of the corresponding gene, leading to the absence of its detection. Therefore a positive PCR shows the presence of the virulence genes but a negative PCR does not point the absence of the corresponding operon[8].

Our results showed a higher frequency of *fimH* compared with the rest of the genes, which may indicated an essential role of the virulence genes in *Escherichia coli* causing UTI. The results for prevalence agree with published reports, which emphasize the predominance of fimbriae type 1 among the UPEC strains[3]. Concerning the frequency of P fimbriae, our results were in correspondence with those of many studies, indicating that among patients with acute pyelonephritis and cystitis, 80% and 30% respectively, possess P fimbriae. A substantial positive correlation was found for simultaneous presence of *sfa* and *pap* which were found together in 15% of the strains[9,14]. Moreover, an important role of *pap* adhesion genes in the pathophysiology of pyelonephritis caused by *Escherichia coli* has been reported in various studies[8]. The presence of

Table 3: The redundancy of uropathogenic *Escherichia coli* virulence factors isolated from patients of Karaj Shahid Rajaii Hospital.

Total	Negative	*aer*	*hly*	*sfa*	*pap*	*fimH*	Gene / Method
100	10	57	47	32	46	73	PCR

hemolysin was related to tissue damage and the prevalence of aerobactin which confer the ability to bind iron, among our isolates was lower than those reported by other investigators[4]. Prevalence of these genes vary according to the phylogenetic groups, clinical conditions of host and geographical localization[9,13]. Prevalence of *aer* was similar to those found in other studies, although a large variation in gene frequencies has also been observed[12]. In the study by using PCR we shown that the frequency of adhesins of UPEC strains, *fimH* and *pap* were more common and *hly*, *aer*, *sfa* similar to some studies and at the same time lower than some[7,9]. The patients who had *fimH*, probably suffered from cystitis and descending infection or at least they are in such a field there was an infection. On the contrary the patients who had *pap*, probably suffered from pyelonephritis and ascending infection or at least they are in such a field there was an infection. In patients who had also *sfa*, *hly* or *aer*, it is likely that there is already a primary sepsis. In the case of UPEC strains that did not have any virulence factors, can be said, all of them were have been related to normal flora of the gastrointestinal tract or may be asymptomatic bacteriuria (ABU).

Our results showed that the UPEC strains isolated in Iran have a different virulence profile compared with other studies and it seems that the virulence of UPEC strains depends on the regional geography and climate. It is believed that the epidemiology and prevalence of UPEC strains virulence factors among Iranian UTI patients are different from other countries. Perhaps some factors such as customs, food diets, public health, and even methods of sampling have great rules in prevalence of virulence genes in UPEC strains[7].

Conclusion

We showed that the characterization of *Escherichia coli* strains isolated from UTI play an important role on developing our knowledge regarding their virulence genetic determinants. Further studies are needed to identify *Escherichia coli* virulence factors responsible for UTI and to determine physiopathology of these infections to consider possible prevention measures. Along with the detection of virulence factors of UPEC strains, serotyping is useful for understanding of relationship between virulence factors. Also, in vivo investigations will be required for authenticity of these results.

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