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Detection of Some Virulence Factors of *Escherichia coli* Isolated from Urinary Tract Infection Isolated of Children in Shahrekord Iran by Multiplex PCR

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Abstract: *Escherichia coli* is considered to be the main causative agent of urinary tract infections (UTIs). The aim of this study was to determine the occurrence of some virulence genes among a hundred *Escherichia coli* isolates obtained patients with urinary tract infection (UTI) refer to medical clinical laborator's Shahrekord, Iran. In this investigation we isolated *E. coli* strains from urine samples of patients with UTI during the period of April 2011 to September 2011and studied them for the presence of the virulence genes by multiplex PCR.A total of 100 *E.coli* strains were isolated, the prevalence of genes such as: pyelonephritis associated pili (*pap* genes), S-family adhesions (*sfa* gene), type1fimbriae (*fim*Hgene*)*, s, aerobactin (aer *gene*) and pyelonephritis isolates compared (*pic* gene) among the isolated strains was:40%, 30%, 30%, 12%, 4%.

Key word: Detection % *Escherichia coli* % Virulence Factors % Multiplex PCR

INTRODUCTION

Urinary tract infections (UTIs) are inflammatory disorders caused by abnormal proliferation of pathogens in the urinary device, while causing changes in the normal functioning of the kidneys and urinary tract [1]. UTI is a major public health problem, being one of the most common infectious diseases encountered in all ages and is one of the most important causes of morbidity and mortality [1]. *Escherichia coli* the most frequent urinary (UTIs) pathogen isolated from 50% - 90% of all uncomplicated urinary tract infections.

The severity of UTI produced by *Escherichia coli* is amplified by the existence of a wide range of virulence factors conferring the uropathogenicity of these strains [1]. The generally accepted hypothesis today is that uropathogenic *E.coli*strains (UPEC) evolved from non-pathogenic strains by acquiring new virulence factors by accessory DNA horizontal transfer often organized into "clusters" (pathogenicity islands) located at chromosomal or plasmidial level [1]. Most of these virulence factors are adherence factors that facilitate the colonization of sites such as the small intestine and urethra, or toxin and effector proteins that affect different host processes [2, 3]. Among these factors, adhesins, aerobactin have been described in previous studies. Analysis of the prevalence of virulence factors as compared to commensal *E.coli* and among those causing different UTIs has indicated a greater virulence potential of such disease causing strains [4].

In the late 1970s it was recognized for the first time that *E.coli* strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of Mannose 2 and this was mediated mainly by

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fimbriae. Subsequently an array of virulence factors have been proposed as virulence markers for uropathogenic isolates of *E.coli* [5].

Bacterial adherence to uroepithelial cells is an essential stage for the initiation and development of UTI. This process allows bacteria to resist the flushing action of the urine flow and bladder emptying, promoting bacterial persistence and activation of the host signaling pathways.

Urovirulence factors of *E. coli* analyzed by molecular methods are useful markers for detection of uropathogenic *E coli* strains [6]. Some virulence factors such as S fimbriae (*sfa*), fimbrial adhesion I (*afaI*), haemolysin (*hly*), cytotoxic necrotising factor 1 (*cnf-1*),aerobactin (*aer*),type1fimbriae (*fim)*, P fimbriae (*pyelonephritis-associated pili*) and chu (hemin uptake system) playimportant roles in the pathogenicity of *E.coli* strains by overcoming host defense mechanisms to cause the disease [6]. It was shown that *afa*, *pap* and *sfa* are predictors of cystitis and/or pyelonephritis [7].

The aim of this study determines some virulence factors in UPEC *E.coli* isolated from urinary tract infections.

MATERIALS AND METHODS

A total of 100 *E.coli* strains during the period of April to September 2011were collected from urine samples of patients refer to medical clinical laborator's Shahrekord, Iran. Bacteria were identified using conventional microbiological methods. The bacteria were maintained in TSB/glycerol at -70°C. These sampleswere processed on MacConkey agar (OxoidLtd.,UK)and were incubated at 37°C overnight. The identification of Gram-negative bacteria was performed by standard biochemical methods (triple sugar iron, urease test, IMViC tests, indole test, catalase test) [8].

DNA Extraction: Bacterial DNA was extracted with the phenol-chloroform method as previously described by Sambrook *et al*. Briefly, the bacteria, grown on nutrient or EMB-agar plates for overnight at 37°C, were suspended in Tris-EDTA buffer (10 mMTris-HCI and 1 mM EDTA) and centrifuged at 2000g for 10 min. Following centrifugation, the pellet was incubated with Tris-EDTA buffer and proteinase K (Fermentas) for overnight at 55 °C. The following day, the DNA was extracted by phenol and chloroform extraction method and suspended in Tris-EDTA buffer [9]. This product was stored as DNA template at -20°C until they were used in the PCR.

Table 1: Primers used in the study for the amplification of genes encoding *pap*, *sfafim*H, *aer* and *pic* genes.

No	Primer Oligonucleotide sequences (5'-3')	Size of amplicon
1	*pap* F GACGGTGTACTGCAGGGTGTC	
	pap R ATATCCTTTCTGCAGGGATGCAA	328 Oliveira
2	*sfa* F CTCCGGAGAACTGGGTGCATCT	410 Oliveira
	*sfa*R CGGAGGAGTAATTACAAACCTGC	
3	*fimH*F AACAGCGATGATTTCCAGTTTGTGTG	465 romanian
	*fimH*R TTGCGTACCAGCATTAGCAATGTCC	
4	*aer* F:AAACCTGGCTTACGCAACTGT	269 bp bladder
	*aer*R:ACCCGTCTGCAAATCATGGAT	
5	*pic* F GGAAGTGACAGGGCATTTGT	352 punjab
	pic R GTGTACCGCTCAGGGTGATT	

PCR Assay: A Multiplex polymerase chain reaction (PCR) was used for the amplification of genes encoding pyelonephritis associated pili (*pap* genes), S-family adhesions (*sfa* gene), type1fimbriae (*fim*Hgene), aerobactin (aer *gene)*and pyelonephritis isolates compared (*pic* gene). The detail of the primers used in the study is given in Table 1. (5).

The PCR assay was carried out in a total volume of 50 µL of mixture containing PCR buffer 10×, 1.5 mM of MgCl2, 250 µM of each of deoxynucleoside triphosphates, 0.5 µM of each of the virulence gene-specific primers, 1.5U of *Taq*polymerase (Sigma), and 5 µL of template DNA. The amplification conditions included 32 cycles of a denaturation step at 94°C for 30 s, primer annealing at 54°C for 30 s, and extension at 72°C for 60 s. The extension time was ramped for an additional 3 s per cycle and a final extension step of 5 min at 72°C was performed. The PCR products were analyzed by 1.5% agarose gel electrophoresis, after which the gel was stained with ethidium bromide and photographed.

RESULTS

Virulence factors such as *pap*, *sfa*, *fim* H, *sat* and *pic* of 100 *E.coli* strains isolated from urine samples of patients with UTI (aged 2 years to 57 years) were analyzed by the multiplex PCR, products of which are shown in Figure 1.

Polymerase chain reaction showed that the prevalence of virulent genes ranged from, 40% for *pap,30% for fim* H, 30% for *sfa*, 12% for *ae r*and*4% for pic*.

DISCUSSION

In an attempt to investigate the prevalence of some important virulence factors *pap,sfa*, *fimb*H, *aer* and *pic* in uropathogenic *Ecoli* strains isolated from urine samples

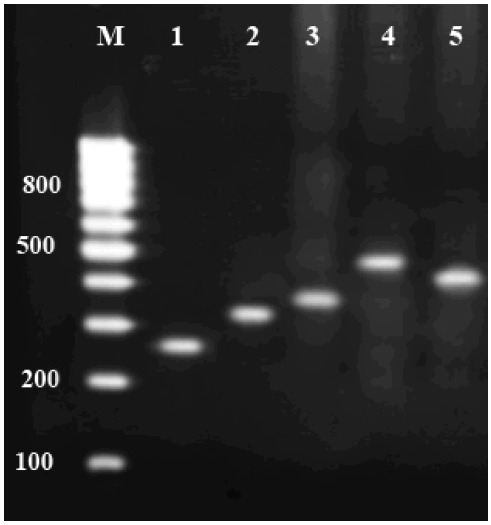

Fig. 1: *Aer, pap, pic, sfa and fim* H genes amplification. (Lane M, Molecular weight marker 100 bp DNA ladder; Lane 1: amplified *aer*gene 269 bp; Lane 2 amplified *pap* gene 328bp; Lane3: amplified *pic* gene 352bp; Lane4: amplified *fim H* gene 465bp and Lane 5: amplified *sfa* gene 410bp

of patients with UTI refer to medical clinical laborator's, Shahrekord city. The PCR results confirmed the presence of virulence factors encoding genes according to their sizes, i.e. *pap* (328 bp), *fim* H (400bp) and *sfa* (410 bp), *aer* (393 bp) and*pic* (269bp) (Fig 1). The current study shows the variable trends of occurrence of various virulence factors (Fig. 2).

We found that *pap, sfa and fimH* operon were the most prevalent virulence factor identified in the strains.The present data indicate the crucial role of these virulence factor in *E. coli*-associated UTI [10, 11]. The distribution of the *sfa* operon (20%) found among the studied strains was also similar to that in the previously reported data.

E.coli is the most common cause of acute *pap*, which is the most serious form of UTI, particularly harmful to newborns and small children. Virulence factors of an *E. coli* isolate act as an epidemiological marker [12]. In the present study, we have demonstrated that the multiplex PCR is a useful method for rapid detection and identification of virulence factors of *E. coli*. Arisoy *et al*., 2006, determined virulence factors of *E.coli* isolated from children; they found that prevalence of *pap, sfa, hly, cnf* and *aer* was: 22.98, 6.21, 1.24, 9.94 and 39.75% respectively [6]. Vazquez *et al*., 1992 in Spain have been reported the prevalence of *pap, aer* and *hly* genes of *E.coli* isolated from blood cultures as 12.50, 61.10 and 20.80%, respectively [13]. Benton *et al*. reported that in patients with spinal injuries, frequencies of *pap, hly* and *aer* were found to be 17, 27 and 39%, respectively [14]. Higher frequency of *aer*, that show *aer* might be one of the main contributors to the persistence of *E.coli*in the intestinal flora in the opposing host defense mechanism.Virulence factors in *E.coli* associated UTI have the crucial role for pathogenicity. Indeed, *pap*gene was found to play more important role in the development of *E.coli* bacteremia in patients with UTI [15]. Moreover, it has recently been shown that the transformation of *E.coli* with *pap* sequences is sufficient to convert it to a more potent host response inducer, with p fimbriae lowering the significant bacteriurea threshold [16] P-pili and S-fimbria (adhesions) are virulence factors responsible for adherence of *E.coli* strains to the extra intestinal host cell surface [17]. *Sat*, as an independent single virulence factor among *E.coli* isolates, is associated with clinical pyelonephritis and cystitis cases. Idress *et al*. [5] reported *sat* and *hly* genes had equal frequency in isolates from pyelonephritis and cystis cases.They reported the combinations of *sat, hly* and *sat, hly, pic* genes were most frequently found in pyelonephritis cases. The prevalence of *pap* is significant only in pyelonephritis. The difference of occurrence in the percentage of the structural adhesions may be due to the different environmental niches prevailing at the two different host sites, as described previously [18]. Farshad *et al*. [18], reported that, prevalence of *pap, cnf, sfa* and *hly* genes among the collected clinical isolates was 30.2, 22.9, 18.8 and 15.6% respectively. This may indicate that *hly* and cnf-1 genes played an important role in causing UTI.

The multiplex PCR is an efficient tool for rapid detection and identification of UTI virulence factors in *E. coli*. In addition, we propose a more significant role for *pap* in generation of UTI.

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