ORIGINAL ARTICLE

Virulence Factors and Antibiotic Resistance in Uropathogenic and Commensal *Escherichia coli* Isolates

Iraj Sedighi[1], Amir Sasan Mozaffari Nejad[2], Ali Amanati[3], Sara Nakhaei[1], Mohamad Yousef Alikhani[4]*

[1]Department of Pediatrics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran, [2]Molecular Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran, [3]Department Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran, [4]Department of Microbiology and Brucellosis Research Center, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

Abstract:

Background: Urinary Tract Infections (UTIs), including cystitis and pyelonephritis, are the most common infectious diseases in childhood. *Aim and Objectives: Escherichia coli* (*E. coli*) account for as much as 90% of the community-acquired and also 50% of nosocomial UTIs. Therefore, the identification of *E. coli* strains and antibiotic resistance patterns is important for both clinical and epidemiological implications. *Material and Methods:* To characterize uropathogenic strains *E. coli*, we studied 100 strains recovered from both urine samples of children aged less than 7 years with community-acquired UTIs and stool samples of healthy children, respectively. *Results:* We assessed Virulence Factors (VFs) and drug sensitivities of *E. coli* isolates. Drug sensitivities of the isolates were 94% (amikacin), 90% (nitrofurantoin), 66% (gentamicin), 56% (cefixime), 40% (nalidixic acid) and 28% (cotrimoxazol). Laboratory tests showed that the prevalence of virulence factors ranged from 18% for hemolysin and P-fimbriae to 2% for type1-fimbriae. Most drug resistance was cotrimoxazole and amikacin was the lowest. P-fimbriae and hemolysin in uropathogenic *E. coli* were more frequent than non-pathogen type of *E. coli*. *Conclusion:* Although amikacin appeared to be the first choice for UTI in children, but nitrofurantoin seems to be practical and could be considered as the selective choice for uncomplicated lower UTIs.

Keywords: *Escherichia coli*; Virulence factor; Drug resistance; Urinary Tract Infection.

Introduction:

Escherichia coli cause several clinical manifestations, including bacteremia, sepsis, meningitis, gastroenteritis and Urinary Tract Infections (UTIs). It is also the most common facultative anaerobic bacteria presented among the gut flora of healthy individuals. *E. coli* species consist of various serotypes, ranging from highly pathogenic to nonpathogenic strains. It has also been noted in a number of studies that certain serotypes of diarrheagenic *E.* coli are more virulent, than other serotypes [1,2].

Uropathogenic *E. coli* is a leading cause of the vast majority of UTIs, and has a wide variety of specific virulence factors such as adhesins and toxins in addition to the common ones [3]. Specific adhesin virulence factors in *E. coli* include Aggregative Adherence Fimbriae (AAF I-IIII), Colonization Factor Antigens (CFA I-III), type 1 fimbriae, P-fimbriae, S-fimbriae, Bundle-forming protein (Bfp), Intimin (non-fimbrial adhesion) and Dr-fimbriae [4]. Specific toxins in *E. coli* are heat Labile Toxins (LT I-II), heat-stable toxins (ST a-b), Shiga-like toxins (Stx 1-2), cytotoxins, endotoxin (Lipopolysaccharide [LPS]) and hemolysin. The common adhesins in uropathogenic *E. coli* are type 1 fimbriae, P fimbriae, S fimbriae, Dr adhesins, afimbrial

adhesins, and the iron-regulated gene A homologue adhesins [5]. Type 1 fimbriae is expressed in both pathogenic and commensal strains, and is considered to be one of the most important VFs involved in the establishment of a UTI mediating extracellular binding to the host urothelium and invasion [6]. Most uropathogenic strains encode type 1 fimbriae for initial attachment to urothelium, which may also facilitate colonization of the microorganism. Infection with type 1-fimbriae *E. coli* might trigger cytokine production and therefore the inflammation. Type 1-fimbriae is most widely distributed among uropathogenic isolates [7]. Type I fimbriae was also found to contribute to biofilm formation of *E. coli* K-12 strain during static growth conditions [7]. P fimbriae act as a major virulence factor associated with pyelonephritis caused by uropathogenic *E. coli* [1]. P fimbriae appear to mediate adherence to urothelial cells in vivo and to establish inflammatory response during renal colonization [8]. P fimbriae may play a role in development of resistance to treatment or relapse of UTI [9]. Of special note, pathogens can develop from commensals isolate by the acquisition of virulence-associated genes located on pathogenicity islands or plasmids and this commensal to pathogen shift in *E. coli* was investigated in other studies [10]. Virulence Factors have been studied widely in the genomes of pathogenic *E. coli*, but little attention has been given to virulence factors in the genomes of commensal members of a species. While determination of virulence factors and antibiotic susceptibility of commensal *E. coli* isolates is important to make the right decision for empiric antibiotic therapy in secondary peritonitis due to intestinal perforation. To understand the virulence factors in commensal isolates of *E. coli*, this study investigated the presence of two markers of virulence factors in *E. coli* isolated from stools of healthy children and the results were compared with those obtained with *E. coli* isolated from the urine of children during UTI. We also tested the antibiotic susceptibility of these isolates according to the presence of different virulence factors.

Materials and Methods

Bacterial isolates

This cross-sectional study conducted between February and October 2012 in Hamadan province, west of Iran. Totally, 100 *E. coli* isolates were studied: 50 *E. coli* isolates selected from stool samples of healthy children with no symptoms of UTI (commensal isolates, were considered as control group), and 50 uropathogenic *E. coli* isolated from children under 7 years of age with community acquired UTIs. Identification of isolates was done using standard microbiological techniques [11, 12].

Haemagglutination tests

Mannose Resistant Haemagglutination (MRHA) with human type A, (3% v/v in PBS in the presence of 2.5% mannose) as the indicator of P fimbriae and mannose sensitive haemagglutination (MSHA) as the indicator of type 1 fimbriae were investigated. *E. coli* was grown on MacConkey's agar plates and inoculated into 5ml of Phosphate Buffered Saline (PBS); then, the species were incubated in pH 7.4 for 5 days at 37° C to obtain fimbriae-enriched *E. coli*. The thin film formed on the surface of PBS was removed and subcultured on Casamino acids-yeast Extract Agar (CEA) and incubated for 18 h at 37°C. Five ml of group 'A' positive human blood was obtained from the blood biobank and added to the same amount of Alsever's solution. In this way, the blood sample was washed three times with Alsever's solution

and 3% erythrocyte suspension was prepared with PBS. The *E. coli* colonies were grown on CEA medium mixed on a VDRL slide in PBS to form a milky suspension. An equal volume of 3% suspension of erythrocytes was added and gently mixed.

The slide was rotated manually for 3-5 min and was sought for macroscopic haemagglutination within 10 min. To determine MRHA, the colonies from CEA were emulsified on a slide in PBS and a drop of 2.5% mannose was added. Equal volume of 3% suspension of erythrocytes was added to this mixture and gently mixed. The slide was rotated for 3-5 min and observed for HA. Haemagglutination was designated as MRHA if HA was observed with and without D-mannose to the same degree and MSHA, if HA was inhibited in presence of D-mannose [13].

Haemolysin Production

Haemolysis activity was shown by the presence of a zone of lysis around or beneath bacterial colonies after overnight incubation on 5% sheep blood agar plate [14].

Antimicrobial Susceptibility Testing

Susceptibility testing for amikacin (30 µg), nitrofurantoin (300 µg), gentamicin (10 µg), cefixime (5 µg), nalidixic acid (30 µg) and cotrimoxazole (25 µg) (MAST, Merseyside, UK), was done on Mueller Hinton agar by disc diffusion method using the Clinical and Laboratory Standards Institute (CLSI) guidelines [15-17]. *Escherichia coli* ATCC 25922 was used as quality control in all assays.

Statistical analysis

Statistical analysis was performed using two-tailed Fisher or $\chi 2$ tests with SPSS software (version 16). P value of < 0.05 was considered to indicate statistical significance.

Results:

Distribution of Virulence Factors in Commensal and Uropathogenic *E. coli* Isolates

In uropathogenic group γ-hemolysis found in 41 (82%) of isolates, and in 9 isolates (18%) β-hemolysis was discovered (Table1). Among total specimen, ten isolates showed β-hemolysis, one of them was in control group and the remaining 9 isolates were in uropathogenic group. Accordingly, uropathogenic *E. coli* had greater tendency to β-hemolysis than isolates in control group, which also had statistically significant difference (P = 0.008). Hemolysis in these specimen, indicated the presence of either P or type 1 fimbriae. Agglutination also analyzed as a virulence factor between two groups (Table 2). Data in Table 2 show categories based on MRHA or MSHA and agglutination negative isolates. Results of MRHA or MSHA column as an agglutination positive isolates (included type 1 and P fimbriae) were combined and analyzed versus agglutination negative isolates by Pearson Chi-Square test ($\chi 2$=1.19, df =1; P =0.275). So, agglutination had no significant difference among the isolates in this study. Also, overall incidence of positive agglutination was 20% (10 isolates) in uropathogenic group, whereas 18% (9 isolates) were MRHA and 2% (only one isolates) were MSHA. Thus, from all uropathogenic *E. coli* isolates which were analyzed 2% had type 1 fimbriae and 18% had P fimbriae. The differences between these virulence factors in uropathogenic group were statistically significant (P = 0.002). Among control group, 12% had positive agglutination, whereas 8% (4 isolates) were MRHA and 4% (2 isolates) were MSHA (Type 1 fimbriae 2% and P fimbriae 8%). Differences between these virulence factors were not statistically significant in control group (P = 0.294). Taken together, P

fimbriae were the most common virulence factors in our study and also had greater number in uropathogenic group than control group.

Antimicrobial Susceptibility

According to distribution of virulence factors in commensal and uropathogenic *E. coli* isolates antibiotic susceptibility testing was conducted. The hemolysis was another virulence factor which was in these isolates. The our results showed that the hemolysis production in *E. coli* correlates with enhanced ability of microorganism in attachment to uroepithelium and so resistance against the bacterial killing process. Isolates with positive (known as β hemolysis) and negative (γ hemolysis) hemolysis were analyzed for antibiotic susceptibility patterns.

The resistant and intermediate isolates were combined and considered as non-susceptible isolates. Nitrofurantoin, amikacin, cotrimoxazol, nalidixic acid, cefixime and gentamicin discs were used on for antibiotic susceptibility testing, due to common prescription of these agents in our region. The pattern of susceptibility to these agents is summarized in Table 3. Drug sensitivy of isolates with β hemolysis was found as: 100% (amikacin), 90% (nitrofurantoin), 70% (gentamicin), 60% (cefixime), 80% (nalidixic acid) and 40% (cotrimoxazole). As shown in Table 3, no significant differences were observed between hemolysis positive and hemolysis negative isolates except for nalidixic acid, which showed statistically significant difference among two mentioned groups (*P* value 0.014). Among β hemolysis positive isolates, amikacin and cotrimoxazol had the lowest and greatest resistance, respectively. We also investigated the antibiotic susceptibility according to the presence of agglutination in all *E. coli* isolates. Susceptibility pattern of isolates with positive agglutination was as: 93% (amikacin), 87% (nitrofurantoin), 68% (gentamicin), 75% (cefixime), 18% (nalidixic acid) and 25% (cotrimoxazole). Among agglutination positive isolates amikacin and nalidixic acid had the lowest and greatest resistances, respectively.

The resistant and intermediate isolates in each group (with positive and negative agglutination) were combined and analyzed as non-susceptible isolates and showed that no significant difference exist between the investigated antibacterial agents except for nalidixic acid (P=0.048) (Table 4).

According to our results, nitrofurantoin was the most sensitive agent just after amikacin in agglutination positive isolates and those with positive hemolysis.

Table 1: Incidence of Hemolysis in *E. coli* Strains

Hemolysis type	Uropathogenic E. coli Number (%)	Enteric isolates E. coli Number (%)
γ-Hemolysis	41 (82)	49 (98)
β-Hemolysis	9 (18)	1 (2)
Total	50 (100)	50 (100)

Analyzed by Pearson Chi-Square (X^2 = 7.111, df = 1; P =0. 008)

Table 2: Incidence of Mannose Resistant and Mannose Sensitive Haemagglutination in *E. coli* Strains

Hemolysis Type	Mannose Resistant Haemagglutination Number (%)	Mannose Sensitive Haemagglutination Number (%)	Without Agglutination Number (%)
Uropathogenic E. coli	9 (18)	1 (2)	40 (80)
Enteric isolates E. coli	4 (8)	2 (4)	44 (88)
Total	13 (13)	3 (3)	88 (88)

2nd and 3rd columns (type 1 and P) have been combined and analyzed by Pearson Chi-Square (X2=1.19, df=1; P=0.275)

Table 3: Susceptibility to Common Antimicrobial Agents among the Isolates showing γ and β Hemolysis Uropathogenic and Enteric *E. coli* Strains

Agent	γ-Hemolysis			β-Hemolysis			P value
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	
Nitrofurantoin	4 (4.4)	6 (6.7)	80 (88.9)	0 (0)	1 (10)	9 (90)	p>0.99
Amikacin	1 (1.1)	2 (2.2)	87 (96.7)	0 (0)	0 (0)	10 (100)	p>0.99
Cotrimoxazole	61 (67.8)	3 (3.3)	26 (28.9)	6 (60)	0 (0)	4 (40)	p>0.482
Nalidixic acid	49 (54.4)	8 (8.9)	33 (36.7)	2 (20)	0 (0)	8 (80)	P=0.014
Cefixime	32 (35.6)	4 (4.4)	54 (60)	4 (40)	0 (0)	6 (60)	p>0.99
Gentamicin	9 (10)	8 (8.9)	73 (81.1)	1 (10)	2 (20)	7 (70)	P=0.414

Resistance and Intermediates have been combined and the Fisher Exact test has been used

Table 4: Susceptibility to Common Antimicrobial Agents in Uropathogenic and Enteric *E. coli* Strains showing Positive or Negative Agglutination

Agent	Agglutination Positive			Agglutination Negative			P value
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	
Nitrofurantoin	0 (0)	2 (12.5)	14 (87.5)	4 (4.7)	5 (6)	75 (89.3)	p>0.99
Amikacin	1 (6.2)	0 (0)	15 (93.8)	0 (0)	2 (2.4)	82 (97.6)	P=0.411
Cotrimoxazol	12 (75)	0 (0)	4 (25)	55 (65.5)	3 (3.5)	26 (31)	P=0.711
Nalidixic acid	12 (75)	1 (6.3)	3 (18.8)	39 (46.4)	7 (8.4)	38 (45.2)	P=0.048
Cefixime	4 (25)	0 (0)	12 (75)	32 (38.1)	4 (4.8)	48 (57.1)	P=0.181
Gentamicin	2 (12.5)	3 (18.7)	11 (68.8)	8 (9.5)	7 (8.4)	69 (82.1)	P=0.303

Resistance and Intermediates have been combined and the Fisher Exact test has been used

Discussion:

UTIs are among the most common infections in infants and neonates. In a previous study by Kausar *et al*, during 2007-2009 in Iran, high rates of resistance to tetracycline, ampicillin, and nalidixic acid were observed among the isolates. They concluded that *E. coli* was the most common bacteria causing UTI and showed a high rate of resistance against many commonly prescribed antimicrobial agents. They concluded that determining the antimicrobial sensitivity can be helpful for consultant doctors in selecting an appropriate treatment for patients suffering from UTI, and also to reduce the complications related to serious UTI in young children [18].

Moreover, one of the important characteristics of uropathogenic *E. coli* is its ability for agglutination of human red blood cells in the presence of mannose. In the current study, we investigated the relationship between the virulence factors of various *E. coli* species and their relationship to the antibiotic resistance. In the present study virulence factors were detected in a 12% of commensal isolates although this was lesser than the percentage seen for uropathogenic isolates (20%). The P fimbriae and fimbriae type 1 had different distribution in commensal and uropathogenic *E. coli* isolates, with significantly higher percentage in uropathogenic isolates. Also, Gulsun and colleagues revealed that in patients with recurrent UTI, MRHA was more common in pathogenic *E. coli* versus nonpathogenic *E. coli*. These researchers proposed that concomitant presence of MRHA and MSHA is associated with increase virulence in *E. coli* [19]. The other survey by Kausar *et al.* (2009), *E. coli* was isolated from symptomatic patients with UTI and compared with enteric isolates from apparently healthy individuals and showed that of pathogenic *E. coli* 21%, 30% and 36% presented with hemolysin, MRHA and MSHA, respectively. In contrast, in the isolates from controls group 16%, 4% and 8% showed hemolysin, MRHA and MSHA, respectively. Hemolysin production had no significant difference between two groups. They reported significant differences between UTI and control group for MRHA. Their results revealed that 80% of pathogenic *E. coli* species, exhibited one or more virulence factors [18].

The pattern of virulence factors among pathogenic *E. coli* investigated by Jalali *et al*. [20]. In his study among 173 isolates of *E. coli* samples in adults with UTI, about 27% had fimbriae type 1, 23% P fimbriae and 23% possessed both of them. Besides, the incidence of MSHA and MRHA estimated about 20% [20]. According to our results, β hemolysis was greater in uropathogenic *E. coli* than the control group and in fact, uropathogenic strain more commonly had hemolysin. In contrast, commensal isolates had greater tendency to γ hemolysis. However, this difference was not statistically significant. Our results also emphasis that hemolysin is one of the significant virulence factor in uropathogenic *E. coli* as previously reported by other investigators [21-22]. In our study, among the isolates with β hemolysis, significantly more common in uropathogenic isolates, 100% were sensitive to amikacin. This is while just 96.7% of commensal *E. coli* isolates harbouring this virulence factor were sensitive to amikacin. In term of agglutination, in the isolates with positive reaction, 93.8% were sensitive to amikacin, whereas, isolates with negative agglutination, 97.6% were sensitive to amikacin.

Conclusion:

In conclusion, the results of this study suggested that P fimbriae, fimbriae type 1 and hemolysin of *E. coli* are common among uropathogenic *E. coli*, that both help in adhering to uroepithelial cells. Based on our findings in this study, although amikacin appeared to be the first choice for UTI in children, nitrofurantoin seems to be practical and could be considered as the selective choice for uncomplicated lower UTIs.

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[*]***Author for Correspondence:*** *Mohamad Yousef Alikhani Department Microbiology and Brucellosis Research Center, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran, E-mail: asmozafarinejad@yahoo.in, alikhani43@yahoo.com Tel: +98-8138380755.*