Original Article

Microbial Susceptibility, Virulence Factors, and Plasmid Profiles of Uropathogenic Escherichia coli Strains Isolated from Children in Jahrom, Iran

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Abstract

Background: Urinary tract infections (UTIs), including cystitis and pyelonephritis, are the most common infectious diseases in childhood. *Escherichia coli* (*E. coli*) accounts for as much as 90% of the community-acquired and 50% of nosocomial UTIs. Therefore, identification of *E. coli* strains is important for both clinical and epidemiological implications. Understanding antibiotic resistance patterns and molecular characterization of plasmids and other genetic elements is also epidemiologically useful.

Methods: To characterize uropathogenic strains of *E. coli*, we studied 96 *E. coli* strains recovered from urine samples of children aged 1 month to 14 years with community-acquired UTIs in Jahrom, Iran. We assessed virulence factors (VFs), drug sensitivities, and plasmid profiles.

Results: Drug sensitivities of the isolates were: 19.8% (ampicillin), 24% (trimethoprim-sulfamethoxazole), 29.2% (tetracycline), 75.5% (nalidixic acid), 80.4% (cefixime), 84.6% (gentamicin), 91.4% (ciprofloxacin), 96.8% (nitrofurantoin), 96.8% (amikacin) and 100% (imipenem). Totally, 76 isolates harbored plasmids with an average of 5.5 plasmids (range: 1 – 10) in each strain. Plasmid profiling distinguished 22 different E. coli genotypes in all isolates that ranged in similarity from 50% to 100%. PCR showed that the prevalence of virulence genes ranged from 15.62% for hly to 30.2% for pap.

Conclusion: These data mandate local monitoring of drug resistance and its consideration in empirical therapy of *E. coli* infections. Plasmid analysis of representative E. coli isolates also demonstrates the presence of a wide range of plasmid sizes, with no consistent relationship between plasmid profiles and resistance phenotypes. Plasmid profiles distinguished more strains than did the antimicrobial susceptibility pattern.

Keywords: *E. coli*, plasmid, UTI, virulence genes

Introduction

*E*scherichia coli (E. coli) is one of the most important causes of community-acquired and human nosocomial infections. The organism is therefore of clinical importance and can be isolated from various clinical specimens.[1] Urinary tract infections (UTIs), including cystitis and pyelonephritis, are the most common infectious diseases in childhood. *E. coli* accounts for as much as 90% of the community-acquired and 50% of the nosocomial UTIs.[2,3]

The pathogenic potential of *E. coli* strains is thought to be dependent on the presence of virulence factors (VFs),[4] which are located on large plasmids and/or in particular regions, called 'pathogenicity islands' (PAIs), on the chromosome.[5,6] Identification of *E. coli* strains is important for both clinical and epidemiological implica-

tions. Understanding antibiotic resistance patterns and molecular characterization of plasmids and other genetic elements is also epidemiologically useful. Antibiotic susceptibility is reported to be dynamic in bacteria, and it differs according to time and environment.[7] Therefore, there is a need for periodic screening of common bacterial pathogens to determine their antibiotic susceptibility profiles in different communities.[1] Comparing plasmid profiles is a useful method to assess the possible relatedness of individual clinical isolates of a particular bacterial species for epidemiological studies.[8]

The present study isolated *E. coli* strains from clinical samples of patients with UTIs who resided in Jahrom, a city in southern Iran. Strains were isolated by culture methods and characterized by the appropriate biochemical, serological, and antibiogram tests. In this study, we performed molecular techniques such as plasmid profile analysis and PCR. This study also investigated the reliability of drug sensitivity patterns and plasmid profiles in the discrimination of *E. coli* strains isolated from UTI epidemics.

Materials and Methods

Patients and bacterial isolation

E. coli strains were isolated from urine samples of children aged 1 month to 14 years, who presented at Motahari Hospital, Jahrom,

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Accepted for publication: 14 September 2011

Table 1. Antibiotic sensitivity of *E. coli* strains isolated from children with UTI

Antibiotic	Sensitivity n (%)
Ampicillin	19 (19.8)
Trimethoprim- Sulfamethoxazole	23 (24)
Tetracycline	28 (29.2)
Nalidixic acid	72 (75.5)
Cefixim	77 (80.4)
Gentamicin	81 (84.6)
Ciprofloxacin	88 (91.4)
Nitrifurantoin	93 (96.8)
Amikacin	93 (96.8)
Imipenem	96 (100)

Table 2. Prevalence of virulence genes in *E. coli* strains isolated from different groups of children with UTI.

Virulence genes		Clinical findings (%)		Kidney ultrasound (%)		Sex (%)	
		Pyelonephritis	Cystitis	Normal	Abnormal	Male	Female
pap	+ (%)	66.7	33.3	62.5	37.5	41.7	58.3
	- (%)	46.5	53.5	54.5	45.5	33.3	66.7
sfa	+ (%)	62.5	37.5	33.3	66.7	33.3	66.7
	- (%)	50	50	59.3	40.7	35.9	64.1
cnf-1	+ (%)	63.6	36.4	16.7	83.3	35	65
	- (%)	48.7	51.3	72.4	27.6	35.7	64.3
h ly	+ (%)	85.7	14.3	100	0	50	50
	- (%)	47.1	52.9	61.8	38.2	333.3	66.7

Iran. *E. coli* isolates were identified by standard methods.[9] The exclusion criteria were recent antibiotic use during the past 28 days and nosocomial infections, defined as infections 48 h post-admission or within 4 weeks following a previous discharge. Positive urine cultures were defined by the growth of a single colony morphotype with counts > 10^5 colony forming unit/ml.

Susceptibility testing

Susceptibility of all the isolates to different antibiotics was determined by the disk diffusion method, as recommended by the National Committee for Clinical Laboratory Standards.[10] Commercial antimicrobial disks (Mast Co., UK) used in this study were: ampicillin (10 µg), nalidixic acid (30 µg), cefixime (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), amikacin (30 µg), and imipenem (10 µg). *E. coli* ATCC 25922 was used for quality-control purposes.

Preparation of bacterial DNA

DNA to be amplified was extracted from the whole organisms by boiling. Bacteria were harvested from 1.5 ml of an overnight Luria-Bertani broth culture, suspended in sterile distilled water, and incubated at 95ºC for 10 min. Following centrifugation of the lysate, the supernatant was stored at -20ºC as a template DNA stock. DNA from uropathogenic *E. coli* strain J96 was extracted and used as a positive control in our PCR reaction.

Detection of virulence factors (VFs)

Detection of *pap*, *sfa*, *cnf-1*, and *hly* genes was performed by gene amplification using Multiplex-PCR. The primer sequences were previously reported[11] and obtained from TIB MOLBIOL Syntheselabor GmbH (Berlin, Germany). Descriptions and sequences of the PCR primers used in this study are presented in Table 1. Other enzymes and chemicals were provided by Cinnagen Chemical Company (Tehran, Iran). The amplification steps were accomplished based on methods described by Yamamoto et al. using a thermal cycler (Eppendorf, Germany).[12] Negative control reactions with distilled water were performed with each batch of amplification to exclude the possibility of any contamination. Expected sizes of the amplicons were ascertained by electrophoresis in 1.5% agarose gel with an appropriate molecular size marker (100 bp DNA ladder, MBI, Fermentas, Lithuania).

Plasmid DNA extraction

Plasmid DNA was extracted from *E. coli* strains according to the alkaline lysis method by Brinboim and Doly (1979).[13] Extracted plasmid DNA was separated by horizontal electrophoresis in an 0.8% agarose slab gel in tris-acetate EDTA (TAE) buffer at room temperature at 60 V for 4 h. Using ethidium bromide, the gel was stained after electrophoresis and video images were prepared by a gel documentation system. The molecular mass of the unknown plasmid DNA was assessed by comparing plasmid mobilities with the known supercoiled DNA ladder (Gibco-BRL, England). The Photo Capt Mw program was used to determine the molecular weight of plasmid bands and analyze plasmid profiles.

Analysis of similarity among strains and construction of a dendrogram

Similarities among the isolates as based upon plasmid profiles were analyzed by Numerical Taxonomy and Multivariate Analysis System software (NTSYS-PC ver. 2.02) for dendrogram construction. The matrix of similarity of coefficients was subjected to un-weighted pair-group method analysis (UPGMA) to generate dendrograms using the average linkage procedure.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows, ver.15 (SPSS, IBM, USA). Chi-square was used to evaluate the variables correlation. *P* values less than 0.05 were considered significant.

Results

Patients and E. coli strains

Totally, 96 strains of *E. coli* were isolated from children with UTI, aged 1 month to 14 years (mean 21.8 ± 26.9 months). There were 60 females (62.5%) and 36 males (37.5%). Among patients, 46.6% had cystitis and 53.3% were diagnosed with acute pyelonephritis, which was more prevalent in girls (63.2% vs. 36.4%, *p* = 0.04). Only 37 patients underwent kidney sonography. Fourteen cases had abnormal findings that included reflux, UPJ stenosis, multicystic kidney, and single kidney.

Antibiotic susceptibility analysis

As shown in Table 1, drug sensitivities of the isolates were: 19.8% (ampicillin), 75.5% (nalidixic acid), 80.4% (cefixime), 84.6% (gentamicin), 91.4% (ciprofloxacin), 96.8% (nitrofurantoin), and 96.8% (amikacin). Sensitivity to imipenem was 100%. Multiple resistance to ampicillin, gentamicin, nalidixic acid, and cefixime were seen in 2.1% of the isolates, but no case of multidrug resistance to all drugs was detected. Only 12.5% of the strains were susceptible to all tested antibiotics. The remaining strains were resistant to one or more antibiotics.

Detection of E. coli virulence genes by PCR assay

PCR assay showed that the prevalence of virulence genes ranged from 15.62% for *hly* to 30.2% for *pap*. Of the studied toxin coding genes, *cnf-1* (22.91%) was more prevalent than *hly* (15.62%). For the adhesion coding genes, *pap* (30.2%) was more prevalent than *sfa* (18.75%). There were 67 (69.8%) strains that were negative for the virulence genes.
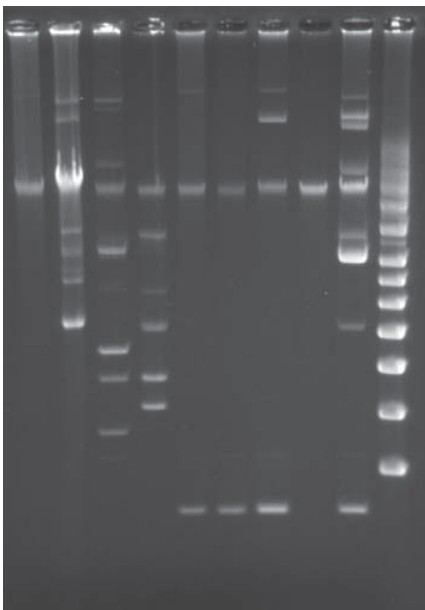

Figure 1. Plasmid patterns of some representative uropathogenic *E. coli* strains.

Plasmid profile analysis

Analysis of plasmid DNA revealed that, totally, 76 isolates harbored plasmids with an average of 5.5 plasmids (range from 1 – 10) in each strain. Figure 1 shows the plasmid patterns of some representative strains of the isolates. Plasmid sizes ranged from 1 to 33 kb in the isolates. The plasmids with the sizes of 4-5 kb were

the most frequent plasmids, and were seen in about 28.94% of the isolates, while plasmids of 11 – 12 kb, 21 – 22 kb, 26 – 27 kb, and 29 – 30 kb were detected in only 1.31% of the isolates.

Genetic similarity among the isolates

The genetic similarities among the 76 *E. coli* strains based on their plasmid patterns are represented by the dendrogram shown in Figure 2.

Discussion

Frequent irrational use of antibiotics changes the intestinal flora, leading to bacterial resistance.[14] In this study we observed a high incidence of antibiotic resistance among the uropathogenic *Escherichia coli* strains. Although resistance to tetracycline was high (70.8%), ampicillin (80.2%) was the most resistant, followed by trimethoprim-sulfamethoxazole (76%). High levels of resistance to tetracycline, ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol and sulphonamide have also been reported in other studies.[15–17] In a previous study in Shiraz, Iran, high levels of resistance to ampicillin (63%), trimethoprim-sulfamethoxazole (48%), and tetracycline (57%) were documented among *E. coli* strains obtained from urine samples.[18] However, the incidence of resistance to these antibiotics was higher in our UPEC strains compared to the Shiraz study. As Jahrom is a small city located southeast of Shiraz, the increase in antibiotic resistance observed in this study could be due to an irrational consumption of antibiotics and food from animals that have received antibiotics, transmission of resistant isolates among people, self-medication, and noncompliance with medication.

No resistance to imipenem was observed in the studied isolates. A high sensitivity of *E. coli* strains to imipenem has been previously reported.[16,18–21] It seems this antibiotic can serve as a medication of choice for the treatment of UTI caused by *E. coli*. However, it should be noted that unlimited use of a medicine can gradually lead to rising antibiotic resistance.

Resistance to nalidixic acid and chloramphenicol in our isolates was lower than that observed in studies performed in other parts of the world.[16,17]

In the present study, it has also been shown that resistance to ciprofloxacin (8.3%), norfloxacin (8.3%), nitrofurantoin (3.1%), and amikacin (3.1%) was low among the UPEC isolates. Shao et al.[22] have shown that amikacin and nitrofurantoin are the most effective treatments in children with UTI in China, which could be explained by the low numbers of prescriptions of these antibacterial agents for UTI. Thus, they could be used as effective therapy for children in our area.

A high incidence of multidrug resistant (MDR) strains was also detected among the present isolates. About 77% were resistant to 3 or more tested antibiotics. The level of MDR among UTI isolates varies from country to country. For example, it was reported to be 7.1% in the USA,[24,25] while 42% of the UPEC isolates in Slovenia in 2006 were MDR.[17] MDR causes major consequences such as empirical therapy of *E. coli* infections as well as possible co-selection of antimicrobial resistance mediated by MDR plasmids. The WHO guidelines recommend trimethoprim-sulfamethoxazole and ampicillin as the first choice for UTI treatment.[26] In contrast, as revealed in the present study, these two antibiotics cannot serve as treatment of choice in our region.
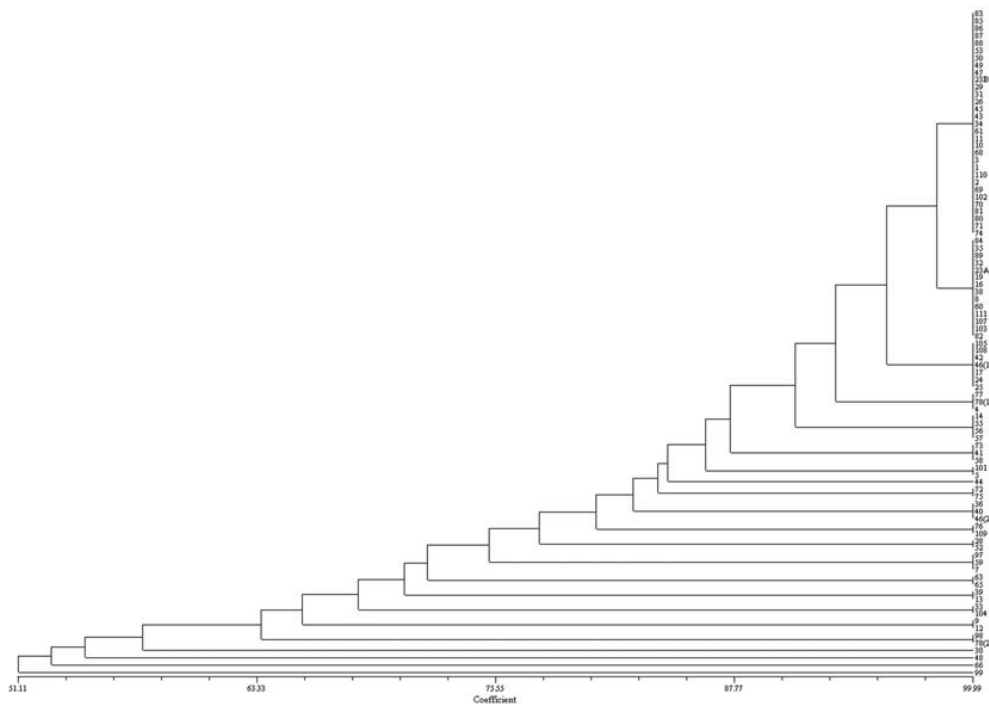
Figure 2. Genetic similarities among 76 *E. coli* strains based on their plasmid patterns.

Antibiotic resistance among bacteria can occur via plasmids. Transmission of specified characterization through plasmids (vertical and horizontal) is better than that through a particular bacterial clone. In this research, to reveal the clonality of UPEC strains isolated from community-acquired UTIs, the plasmid patterns of the isolates were investigated.

The results showed that 76 (79%) of the isolates harbored an average of 5.5 plasmids. Other reported results agreed with our study. Woo-Joo et al. have reported that 87.5% and 72% of UPEC strains carried plasmids.[27] In another study undertaken by Fluit, the prevalence of plasmid in the isolates was 81%, which was also similar to our results.[28]

In the present study, the range of plasmids was 1-10 while Malkawi has reported the numbers of plasmids to be approximately 1 – 6 in *E. coli* strains.[29]

Molecular weights of the plasmids were between 1-33 kb. In a research conducted by Malkawi, the plasmid sizes were from 1.5 – 54 kb.[29] Tsen has reported a range of 2 – 22 kb for plasmid sizes.[30] Danbara et al. have also reported plasmid size variations between 3.9 kb and 50 kb in *E. coli* strains.[31] We detected plasmid weight ranges of 11 – 12 kb, 21 – 22 kb, 26 – 27 kb, and 29 – 30 kb in only 1.31% of the isolates. Those with 4-5 kb were the most frequent plasmids, seen in about 28.94% of the isolates and among the strains resistant to the medicines under the study. These data show that the former plasmids have a lower stability in comparison with 4 – 5 kb plasmids. As ampicillin showed the most resistance, therefore we have suggested that the gene coding for ampicillin resistance could be located on this plasmid. On the other hand, 21% of our isolates have no plasmids, yet they were resistant to a large number of antibiotics. Possibly, some antibiotic resistance genes may not be located in the plasmid but may be on the bacterial chromosome. In order to prove the relationship between the plasmid and its resistance, additional studies such as plasmid curing and transferring of the plasmid to other known bac-

teria should be performed. Similarity among isolates on the basis of the plasmid profile was also analyzed by NTSYS-PC ver. 2.02K software (Figure 2). As seen in the dendrogram, similarities ranged from 50% to 100%. Plasmid profiling could distinguish 22 different *E. coli* genotypes in all isolates named A1-A22. Pattern A1 has included 31 isolates with 100% similarity and pattern A2 has 14 isolates with 100% similarity. It seems that patients with *E. coli* strains with each of these two models of plasmid patterns are likely to obtain the sources of the bacteria from a clone with a high incidence of bacterial gene transfer in the community. According to the data shown in Table 2, plasmid profiles distinguished more strains than did the antimicrobial susceptibility pattern.

Saif and Umolu reported a high prevalence of plasmids in antibiotic resistant *E. coli* strains isolated from animals.[1,32] In Jahrom, most people are in close contact with animals, thus it could be suggested that animals may be a source for antibiotic resistant gene dissemination.

In an attempt to investigate the prevalence of 4 important VFs, *cnf-1*, *sfa*, *pap* and *hly*, in resistant compared to susceptible uropathogenic *E. coli* strains isolated from urine samples of children with UTI, we found that *pap* operon was, as expected, the most prevalent virulence factor identified. Regarding *pap*, pooled results with the present data indicated a crucial role of this virulence factor in *E. coli*-associated UTI.[33,34] 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 bacteriuria threshold.[35] The distribution of the *sfa* operon found among studied strains was also similar to previously reported data. The prevalence of *hly* among the collected clinical isolates also matched those reported by other investigators.[4,35] However, in our study, the *cnf-1* operon was more prevalent than in other studies.[4,11,35] Possibly, the *cnf-1* gene played an important role in UTI in our study.

In conclusion, the high incidence of MDR strains detected among

the present isolates mandate local monitoring of resistance and its consideration in empirical therapy of *E. coli* infections, particularly those which cause UTIs. We found that *pap* operon was, as expected, the most prevalent virulence factor identified. Plasmid analysis of representative *E. coli* isolates also demonstrated the presence of a wide range of plasmid sizes, with no consistent relationship between plasmid profiles and resistant phenotypes. A common large plasmid with a molecular size of 28 kb was responsible for transferring partial resistance. In our study, plasmid profiles distinguished more strains than the antimicrobial susceptibility pattern.

Acknowledgments

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