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Full Length Research Paper

Molecular epidemiology of *Escherichia coli* strains isolated from children with community acquired urinary tract infections

Shohreh Farshad[1*], Mojtaba Anvarinejad[1], Ali Mehrabi Tavana[2], Reza Ranjbar[3], Aziz Japoni[1], Reza Mohammad Zadegan[3] and Abdolvahab Alborzi[1]

[1]Clinical Microbiology Research Center- Shiraz University of Medical Sciences, Nemazi Hospital, Shiraz 71937-11351, Iran.

[2]Health Research Management Center- Baqiyatallah University of Medical Sciences, Tehran, Iran.

[3]Molecular Biology Research Center-Baqiyatallah University of Medical Sciences, Tehran, Iran.

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This paper aimed to study the drug resistance patterns and the reliability of antibiogram and pulsed field gel electrophoresis (PFGE) patterns in epidemiological study of uropathogenic *Escherichia coli* (UPEC) isolated from the children with community acquired urinary tract infection (UTI). Totally ninety *E. coli* strains were isolated from urine samples of children with community acquired UTI and tested for susceptibility to fourteen different antibiotics, using disc diffusion method. To study the molecular epidemiology of these strains, their genomic patterns were determined using PFGE. Forty five patterns of resistance have been recognized for the *E. coli* strains. High prevalence of resistance to ampicillin (80.2%), trimethoprim-sulfamethoxazole (76%), and tetracycline (70.8%) was seen among the UPEC isolates. All the isolates were 100% sensitive to imipenem. In PFGE, sixty five patterns from the genome of *E. coli* strains were observed. Based on drawn dendrogram the sample patterns were divided into two groups. All the samples except two have different clonalities. In conclusions it seems that imipenem, amikacin and nitrofurantoin can serve as drug of choice for the treatment of UTI caused by *E. coli*, respectively. With regard to high differentiation power of PFGE method in comparison with antibiogram and according to the obtained patterns and high diversity of these profiles, no epidemic UPEC was determined in the studied population.

Key words: *Escherichia coli*, urinary tract infection, children, epidemiology, genotyping.

INTRODUCTION

Urinary tract infection (UTI) is a common cause of fever and one of the most common community acquired infections in children (Guidoni et al., 2008). It is estimated that about 8% of girls and 2% of boys have at least one episode of UTI during childhood (Mohkam et al., 2008). UTI is the most frequently diagnosed kidney (pyelonephritis) and bladder (cyctitis) disorders, and approximately 80-90% of all the community acquired UTI cases are caused by *Escherichia coli* strains (Ejrnaes et al., 2006). Cystitis is characterized by dysuria, frequency and urgency of urination and in some cases suprapubic pain and bladder limited infection. Pyelonephritis, an infection of the kidneys with potential for bacteremia, clinically presents with flank pain, fever, nausea, vomiting and malaise (Rasko et al., 2001). The clinical management of UTI is complicated due to the increasing incidence of infections caused by the strains of *E. coli* that are resistant to the commonly used antimicrobial agents (Manges et al., 2001). Increasing rates of resistance among uropathogens have caused growing concern in both developed and developing countries

*Corresponding author. E-mail: farshads@sums.ac.ir. Tel: +98 711 6474264. Fax: +98 711 6474303.

(Dromigny et al., 2005). However, the patterns of resistance are different in various geographic areas (Sahm et al., 2000). Recognition of these patterns in different communities is vital to distinguish uncommon or new patterns. On the other hand particular attention to early diagnosis and treatment of UTI is mandatory in children to reduce the chronic kidney damage and its clinical consequences. However, traditional methods like antibiogram analysis are often based on phenotypic characteristics. These methods are reported to be insufficiently discriminatory, to have poor reproducibility, and to be affected by physiological factors (Alfizah et al., 2004). It has been shown that in order to study the epidemiology of a disease, genotyping methods are much better than the phenotypic characterization. Pulsed field gel electrophoresis (PFGE) typing which is known as gold standard technique is highly effective in molecular epidemiological studies of bacterial isolates such as *E. coli* strains and it is superior to other methods in discrimination of the isolates (Alfizah et al., 2004; Sandt et al., 2006). Therefore, in this study, we first evaluated the susceptibility patterns of uropathogenic *E. coli* (UPEC) clinical strains, isolated from children with UTI in the community of Jahrom, Iran, against fourteen antibiotics in order to arrive at the best therapy for patients and prevent growing drug resistance in this community. Furthermore, we evaluated the molecular epidemiology of *E. coli* strains using PFGE method to investigate reliability of drug sensitivity and PFGE patterns in discrimination of epidemic strains of UPEC isolated from the community under the study.

MATERIALS AND METHODS

Bacterial isolation

During a period of one year from 2007 to 2008, UPEC strains were isolated from urine samples of children aged from one month to fourteen years with UTI who were referred to Motahari hospital, Jahrom, Iran. The isolates were identified as *E. coli* by standard methods (Murray, 1999). UTI diagnosis was established by the hospital physicians based on clinical symptoms and laboratory investigations. Positive urine cultures were defined by a bacterial growth more than 10^5 colony forming unit per ml. As the cases considered in this study were only the patients with community acquired UTI, the exclusion criteria were recent antibiotic use during the last fifteen days and nosocomial infections which were defined as infections noted 48 h after admission or within four weeks after a previous discharge.

Antibiotic susceptibility

Susceptibility of all the isolates to different antibiotics was determined by the disc diffusion methods as recommended by CLSI (2006) with commercial antimicrobial discs (Mast. Co, UK). The used antibiotic discs in this study were ceforoxime (CXM), ceftazidime (CAZ), norfloxacin (NOR), trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), chloramphenicol (CHL), ampicillin (AMP), nalidixic acid (NAL), cefixime (CFM), gentamicin (GEN), nitrofurantoin (NIT), ciprofloxacin (CIP), amikacin (AMK) and imipenem (IPM). *E. coli* ATCC 25922 was used for quality control purposes.

Pulsed field gel electrophoresis

This procedure was designed based on previously reported protocol by Ejnreas et al. (2006) with some modifications. Briefly, the isolates were grown overnight on blood agar plates at 37°C. In order to protect the DNA against breakage and to allow the free flow of lytic solutions, the bacteria were incorporated into agarose plugs as described below. About three loops of bacteria were washed in 1 ml saline to obtain an optical density of 0.7 at wavelength of 610 nm and resuspended in 1 ml tris(hydroxymethyl)aminomethane ethylene (TE) buffer (10 mM Tris HCl [pH 8.00], 100 mM ethylene diamine tetraacetic acid (EDTA) and incubated at 50°C in a water bath for maximum 15 min. Chromosomal DNA was prepared in solid agarose plugs by mixing 1 ml bacterial cell suspension with an equal volume of 2% low melting agarose (Fermentase, Lithuania) followed by incubation overnight at 54°C in lysis buffer (50 mM Tris HCl [pH 8.00], 50 mM EDTA, 1% laurylsarcosine, 1 mg/ml of proteinase K). The DNA plugs were washed four times in TE buffer for 30 min at 50°C and three times in distilled water. One third of each plug was cut and transferred to a tube containing *Xba*I restriction enzyme (Fermentase, Lithuania) according to the manufacture's instruction and remained overnight at 37°C.

DNA preparations were put in the wells of an agarose gel (molecular grade, Amersham Bioscience, Sweden), and covered with 0.5x TBE buffer and then were run in a homogenous electric field (Amersham Bioscience, Sweden). The electrophoretic conditions used were as follows; initial switch time: 5 s, second switch time 20 s, final switch time 40 s, temperature 12°C, run time 33 h, angle 120°, gradient 6 v/cm. In each set 1000 bp lambda ladder (Biolabs, New England) was used as molecular marker. After electrophoresis the gel was stained in etidium bromide and then photographed. Photocapt software was used to determine the molecular weights of the sample profiles. The bands were considered according to the DNA marker and were scored across all the samples. The bands were recorded as number one for present or zero for absent. Consequently, the data set were used to calculate pair-wise similarity coefficient following the Jaccard method. To generate a dendrogram using average linkage procedure, the analysis of the similarity coefficients matrixes were performed using unweighted pair-group method analysis (UPGMA). To calculate correlations among variables, the standardized data matrixes were used. These correlations were subjected to Eigen vector analysis to evince the first three uttermost elucidative principal components. To study the patterns of variations which were observed among the isolates, the three principal components were plotted. The software NTSYSpc version 2.02i (Exeter software, New York) was used to conduct all the numerical analysis.

RESULTS

Bacterial strains and antibiotic susceptibility

Totally ninety strains of *E. coli* were isolated from the urine samples of children with community acquired UTI, aged one month to fourteen years (mean 21.8±26.9 month). Antibiotic sensitivity patterns of the isolates were determined by standard disc diffusion methods and the results have been shown in Table 1.

Among the drugs under the study, AMP, SXT and TET

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

Antibiotic strains	Total resistant N (%)
Ampicillin	77(80.2)
Trimethoprim-sulfamethoxazole	73(76)
Tetracycline	68(70.8)
Chloramphenicol	34(35.4)
Nalidixic acid	24(25)
Cefixime	19(19.7)
Ceforoxime	18(18.7)
Gentamicin	15(15.6)
Ceftazidime	10(10.4)
Ciprofloxacin	8(8.3)
Norfloxacin	8(8.3)
Nitrofurantoin	3(3.1)
Amikacin	3(3.1)
Imipenem	0(0)

have the least antimicrobial effects. No resistance to IPM was seen among the strains. 77% of the isolates were resistant to three or more antibiotics and were designated as multidrug resistant (MDR). Only 8.3% of the strains were fully susceptible to all tested antibiotics. The remaining strains were resistant to one or more antibiotic (Table 2).

Pulse Filed Gel Electrophoresis

Sixty five PFGE profiles were obtained from the genome of *E. coli* strains which were named from p1 to p65 (Figure 1). Majority of the strains showed twelve to thirteen bands and the patterns with eight or nineteen bands had the least percentage. Pattern p2 was the most repeated pattern through the samples (n=5, 5.5%). The frequency of the bands through the strains of UPEC is shown in Table 3. Sample patterns were divided into two groups based on drawn dendrogram (Figure 2). First group contains sixty samples with thirty-seven patterns. In this group fifty samples have the least similarity. The second group contains thirty isolates with twenty-eight patterns, and all the samples except two have different clonalities.

DISCUSSION

The genetic variation seen in the chromosomal DNA of a bacterial species reflects the ability of molecular typing system to distinguish among epidemiological unrelated isolates (Persing et al., 1993). Usually, this variability is high, and differentiation of unrelated strains can be accomplished using any of a variety of techniques (Goering, 1993). However, the factors that enable bacteria to cause infection often are not uniformly distributed within a species. Thus, the organisms most commonly associated with infections often are a smaller subset of the many strains that constitute a species (Musser, 1996). As a consequence, this subset may exhibit relatively little genetic diversity, and it can be difficult to differentiate among strains even with newer molecular techniques. Typing methods are those that characterize the products of gene expression in order to differentiate strains. Properties such as biochemical profiles, bacteriophage types, antigens present on the cell's surface and antimicrobial susceptibility profiles all are examples of phenotypic properties that can be determined in the laboratory. Because they involve gene expression, these properties all have a tendency to vary, based on changes in growth conditions, growth phase, and spontaneous mutation (Tenover et al., 1997).

In this study to find the antibiotic susceptibility patterns of the *E. coli* strains isolated from community acquired UTIs in children to find the best therapeutic pattern and to assay the epidemiological relatedness among the strains, the susceptibility of the strains to fourteen antibiotics were determined. Although resistance to tetracycline was high (70.8%), the most prevalent was resistance to AMP (80.2%) followed by SXT (76%). High level of resistance to AMP has been also reported in Melborn, Australia through the study of children with UTI by Mehr et al. (2004). Mathai et al. (2004) reported resistance to tetracycline, TET, SXT and sulphonamide among the UPEC strains, isolated in southern India (Mathai et al., 2004). In another study by Rijavec et al. (2006) in Ljubljana, Slovenia, a high incidence of antibiotic resistance among the UPEC strains to AMP, TET and CHL was also determined. High level of resistance to AMP (63%), SXT (48%) and TET (57%) among *E. coli* strains from urine samples has been documented in

Table 2. Antibiotic resistance patterns of the UPEC isolated from children with UTI.

Antibiotic resistance pattern	Number
AMP	6
SXT	4
NAL	1
AMP-TET	2
CXM-SXT	1
CFM-AMP	1
AMP-GEN	1
AMP-TET-SXT	13
AMP-CHL-TET	2
AMP-GEN-SXT	1
NAL-TET-SXT	1
NAL-AMP-TET	1
CHL-TET-SXT	1
AMP-GEN-TET-SXT	1
AMP-CHL-CXM-SXT	1
AMP-NIT-TET-SXT	1
AMP-CHL-TET-SXT	12
CFM-AMP-TET-SXT	3
AMP-AMK-GEN-TET-SXT	1
NAL-AMP-CHL-TET-CXM	1
NAL-AMP-CHL-TET-SXT	2
NAL-CFM-AMP-TET-SXT	2
NAL-CHL-TET-CXM-SXT	1
CFM-AMP-TET-CXM-SXT	1
NAL-AMP-GEN-TET-SXT	1
CIP-AMP-GEN-TET-SXT	1
AMP-C-TET-NOR-CXM-SXT	2
AMP-C-TET-NOR-CAZ-SXT	1
NAL-AMP-GEN-CHL-TET-SXT	1
NAL-CIP-AMP-GEN-TET-SXT	1
CFM-AMP-TET-CXM-CAZ-SXT	1
CFM-AMP-CHL-TET-NOR-SXT	1
CFM-AMP-AMK-TET-CXM-CAZ-SXT	1
NAL-AMP-CHL-TET-NOR-CXM-SXT	1
NAL-CIP-AMP-GEN-C-TET-SXT	1
NAL-AMP-GEN- CHL- TET- NOR- CXM-SXT	1
NAL-CFM- CIP- AMP- GEN- C-TET- SXT	1
NAL-CFM- AMP- CHL- TET- CXM- CAZ- SXT	1
NAL-CIP- AMP- GEN- CHL- TET- NOR- SXT	1
NAL-CFM- AMP- NIT- TET- CXM- CAZ- SXT	1
NAL-CFM-CIP- AMP- GEN- CHL- TET- CXM- SXT	1
CFM-AMP- AMK- GEN- TET- NOR- CXM- CAZ- SXT	1
NAL-CFM- CIP- AMP- NIT- CHL- TET- CXM- CAZ- SXT	1
NAL-CFM- CIP- AMP- GEN- TET- NOR- CXM-CAZ-SXT	1
Sensitive	8
Total	90

Shiraz, Iran, too (Japoni et al., 2008). However, the incidence of resistances to these antibiotics was higher in our UPEC strains, compared to their strains.

As Jahrom is a small city located in southeast of Shiraz, Iran, increased antibiotic resistance observed in the present study could be due to an irrational

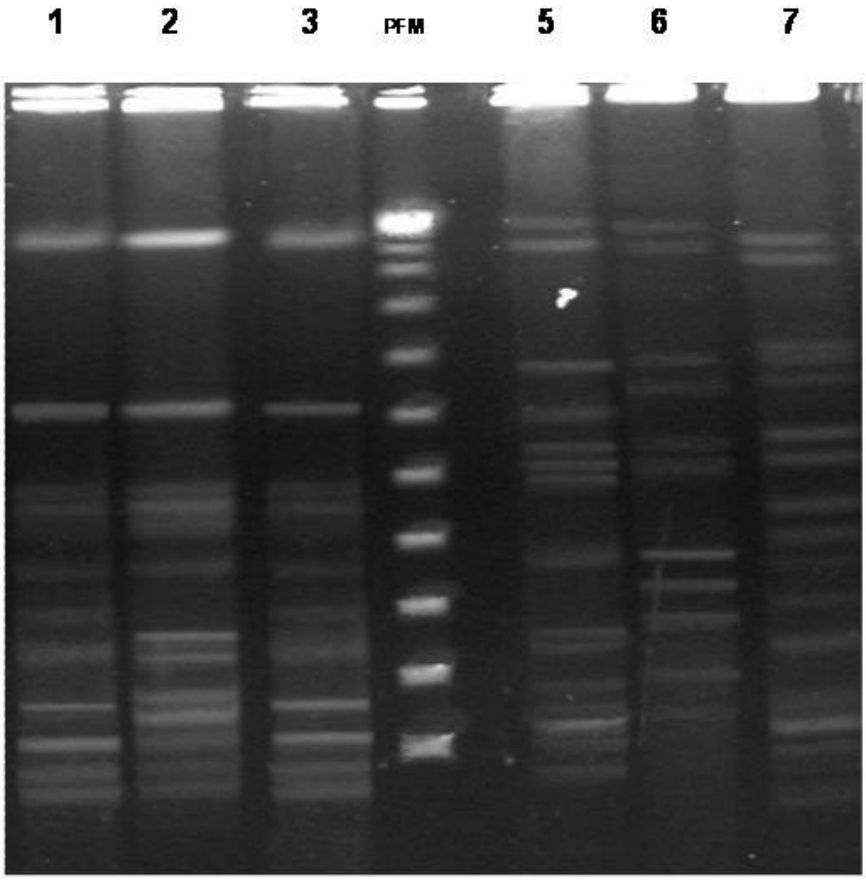


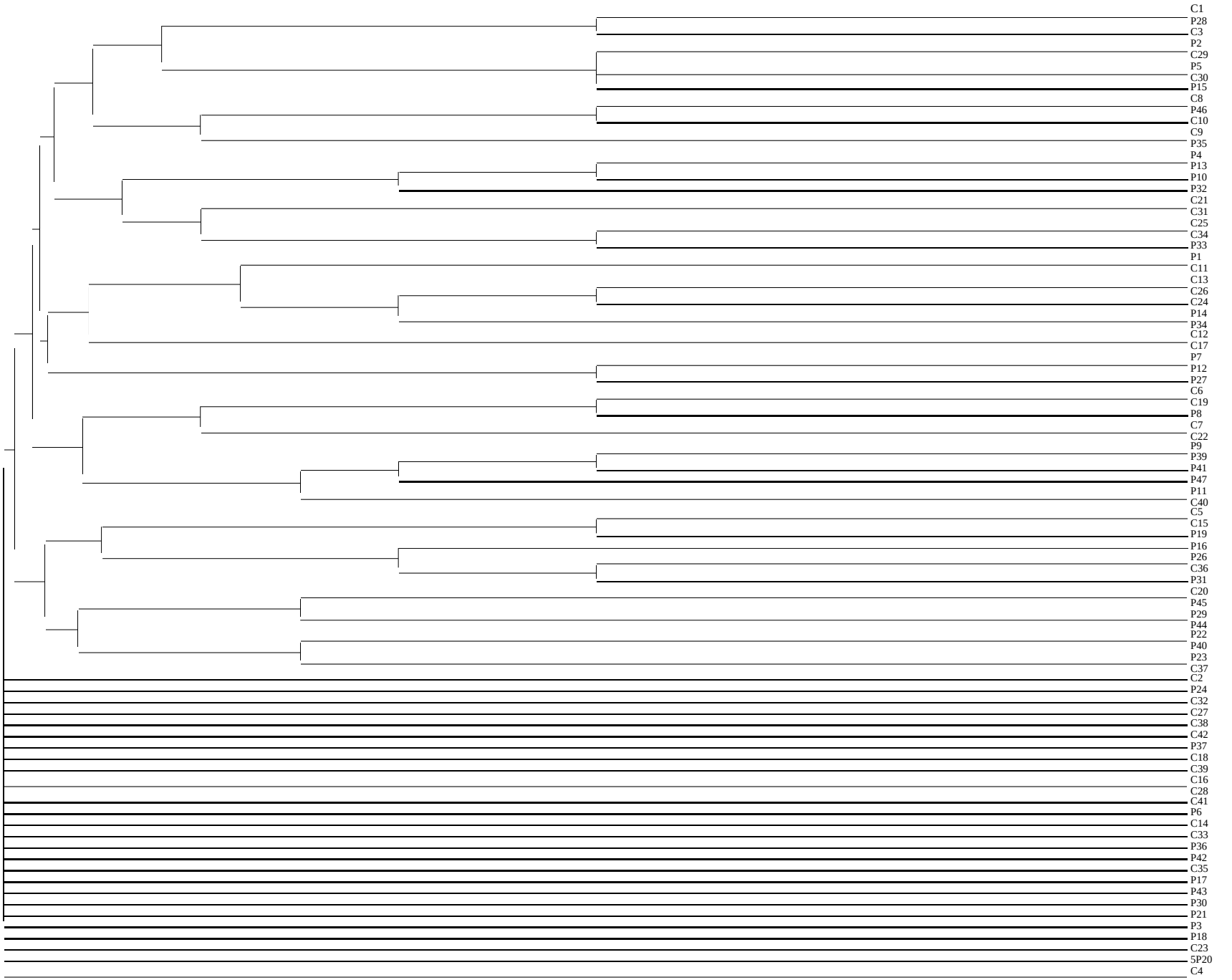
Figure 1. A representative PFGE profile which shows *Xba*I-digested genomic DNA of *E. coli* strains isolated from children with UTI. Lanes 1, 2, 3, 5, 6, 7 *E. coli* strains; Lane 4 is lambda ladder used as molecular size marker (PFM).

Table 3. Numerousness of the bands in PFGE of the strains of UPEC.

Number of the band	Number of *E. coli* strain (%)
1(1.1)	8
3(3.3)	9
5(5.5)	10
12(13.3)	11
21(23.3)	12
23(25.5)	13
8(8.8)	14
6(6.1)	15
7(7.7)	16
3(3.3)	17
1(1.1)	19

consumption rate of antibiotics and frequent unwise use of antibiotics. On the other hand in small cities unfortunately, most cases of UTI are treated empirically and the patients are ignorant and also cannot afford to consult physicians or have a laboratory analysis made.

No resistance to IPM was observed among the studied isolates. The same result was obtained by Adwan et al. (2004). High sensitivity of *E. coli* strains to IPM has also been reported earlier (Goering, 1993; Gulsun et al., 2005; Tariq et al., 2006). It seems that this antibiotic can serve as drug of choice for the treatment of UTI caused by *E. coli*. However, it should be noted that non limited


C1
P28
C3
P2
C29
P5
C30
P15
C8
P46
C10
C9
P35
P4
P13
P10
P32
C21
C31
C25
C34
P33
P1
C11
C13
C26
C24
P14
P34
C12
C17
P7
P12
P27
C6
C19
P8
C7
C22
P9
P39
P41
P47
P11
C40
C5
C15
P19
P16
P26
C36
P31
C20
P45
P29
P44
P22
P40
P23
C37
C2
P24
C32
C27
C38
C42
P37
C18
C39
C16
C28
C41
P6
C14
C33
P36
P42
C35
P17
P43
P30
P21
P3
P18
C23
5P20
C4

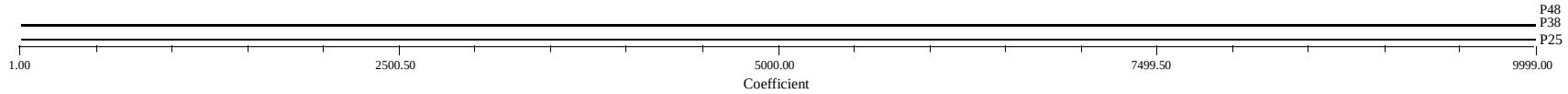


Figure 2. A dendogram showing relationships among *E. coli* isolates from children with pyelonephritis (P) and cystitis(C).

administration of a drug can gradually lead to rising in antibiotic resistance.

Resistance to NAL acid and CHL in our isolates was lower than that observed in some other studies carried out in other parts of the world (Mathai et al., 2004; Rijavec et al., 2006).

In our examination it was also shown that resistance to CIP (8.3%), NOR (8.3%), NIT (3.1%), and AMK (3.1%) was low among the UPEC isolates. Shao et al. (2004) showed that AMK and NIT have the best effect on the treatment of children with urinary tract infection in China. This could be explained by low prescription of these anti-bacterial agents for urinary tract infections in the patients and can still use for therapy of children in determined area.

A high incidence of MDR strains was also detected among our isolates. About 77% of them were resistant to three or more tested antibiotics. While 42% of the UPEC isolates in 2006 in Slovenia were MDR it was reported to be 7.1% in USA (Gulsun et al., 2005; Rijavec et al., 2006, Sahm et al., 2000). Such MDR has serious implications for the empirical therapy of infections caused by *E. coli* and for the possible co selection of antimicrobial resistance mediated by MDR plasmids. The world health organisation (WHO) guidelines indicate TMP/SMX and AMP as first choice for the treatment of UTI (Wolff and Maclennan, 2007). Consequently, it seems that the antibiotic sensitivity patterns to the habitual antimicrobials in Jahrom, Iran recommend against their use as the first choice.

In comparison with antibiotic susceptibility, genotyping methods such as PFGE have shown high discriminatory power for epidemiological investigation (Rudolph et al., 1998). The development of PFGE typing methods based on fingerprinting of bacterial genome has given valuable tools to confirm the relationship among outbreak strains (Alfizah et al., 2004). In current study in the second part, we applied PFGE followed by *Xba*I restriction digestion of chromosomal DNA to determine the genetic relatedness among UPEC isolates.

As shown in Figure 1, we observed sixty-five PFGE patterns based on the drawn dendrogram, while antibiotic susceptibility test defined only forty-five patterns. In previous studies researchers observed a high diversity among the PFGE patterns (Bannerman et al., 1995; Kawamori et al., 2008; Watabe et al., 2008). The best description for these diversities is that the isolates representing the outbreak strains are not the recent progeny of a single (or common) precursor and do not have the same genotypes. On the other hand the high diversity of PFGE patterns observed in this study is not surprising because numerous samples were collected from different parts of community in Jahrom during a period of year.

Using this method in the present study, eight to nineteen bands with the molecular weigh of the 5-660 kb were observed in the PFGE profiles of the strains. Kawamori et al. (2008) and Li et al., (2007) reported nineteen to twenty-four DNA bands with molecular sizes of 30-500 kb, in molecular typing of Japanese *E. coli* isolates. Ejrnaes et al. (2006) reported fifteen to twenty distinct bands with 50-1200 kb molecular weight by PFGE typing of UPEC strains. In Northern Ireland, Watabe et al. (2008) in an epidemiologic study of *E. coli* isolates, observed fifteen DNA fragments using PFGE. These different results show the high diversity of *E. coli* strains in different geographic area. Random genetic events including point mutations, insertion and deletion of DNA can alter PFGE profiles. These events are shown by presence or absence of the determined bands (Goering, 1993; Murray, 1999). However, technical difficulties of laboratory methods can be assumed as another reason for such observed varieties. It also depends on other factors such as; individual person, laboratory setting up and equipments, reagents, interruptions or distractions and unknown reason.

Conclusion

This is the first report of applying PFGE genotyping method to study molecular epidemiology of UPEC infection in Iran. This report revealed that the UPEC strains isolated from the population of children of Jahrom community were not epidemically related. With regard to high diversity of strains, we also could not find a correlation between pattern of PFGE and antibiogram profiles. In these cases, obtaining additional information, such as the use of a second strain typing method or supplementary epidemiological analysis are recommended.

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Abbreviations: UPEC, Uropathogenic Escherichia coli; **UTI,** urinary tract infection; **PFGE,** pulsed field gel electrophoresis; **CXM,** ceforoxime; **CAZ,** ceftazidime; **NOR**, norfloxacin; **SXT**, trimethoprim-sulfamethoxazole; **TET,** tetracycline; **CHL**, chloramphenicol; **AMP**, ampicillin; **NAL,** nalidixic acid; **CFM**, cefixime; **GEN,** gentamicin; **NIT,** nitrofurantoin; **CIP,** ciprofloxacin; **AMK**, amikacin; **IPM**, imipenem; **EDTA**, ethylene diamine tetraacetic acid; **UPGMA**, unweighted pair-group method analysis; **TE buffer,** tris(hydroxymethyl)aminomethane ethylene; **MDR,** multidrug resistant; **WHO,** world health organization.

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