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# Low Distribution of Integrons among Multidrug Resistant *E. coli* Strains Isolated from Children with Community-Acquired Urinary Tract Infections in Shiraz, Iran

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Abstract

Although integrons by themselves are not mobile, due to their presence in plasmids and transposons, they can be transferred horizontally. For these reasons integrons are a major mechanism for the spread and maintenance of multidrug resistance (MDR). This study describes the distribution of integron gene cassette classes in a collection of uropathogenic *Escherichia coli* (UPEC) isolated from children with community acquired urinary tract infection in Jahrom, Iran. *E. coli* strains isolated from urine samples were tested for susceptibility to 14 different antibiotics using the disk diffusion method and for integron classes by RFLP-PCR. Totally 96 strains of *E. coli* were isolated from urine samples. High prevalence of resistance to ampicillin (80.2%), co-trimoxazole ((76%) and tetracycline (70.8%) was seen among the UPEC isolates. All isolates were 100% sensitive to imipenem. Sixteen strains (16.6%) had the evidence of integron sequenceswith the prevalence of 6.25% (n = 6) and 10.41% (n = 10) for *intI1* and *intI2*, respectively. No *intI3* was detected in the isolates. The presence of integrons was significantly associated with resistance to certain antibiotics including gentamicin and ampicillin. Considering the MDR patterns and the low prevalence of integrons among the *E. coli* strains under the study, we suggest that the antibiotic resistance cassettes in these strains presumably are mostly carried on the other transposable elements rather than integrons.

Key words: *Escherichia coli*, antibiotic sensitivity, integron, urinary tract infections

## Introduction

Urinary tract infections (UTIs) are the most common infectious diseases in childhood. As much as 90% of community-acquired and 50% of the nosocomial UTIs are caused by *Escherichia coli* (Svanborg and Godaly, 1997; Vila *et al*., 2002).

As extra intestinal pathogenic *E. coli*, including uropathogenic *E. coli* (UPEC), yearly affects a large proportion of the population; they are a major target of antimicrobial therapy. Increasing antimicrobial resistance among bacteria causing UTI is therefore of great concern. Patterns of increased antimicrobial usage are the main driving force in generating and maintaining resistant bacteria (World Health Organization, 2001). It has been also shown that, once evolved, resistance genes can spread through the world's bacterial populations irrespective of the pattern of antimicrobial use in an area (O'Brien, 2002). Therefore, mechanisms other than selection pressure might exist for maintaining a resistant bacterial pool. Resistance genes are disseminated by plasmids or by transposons and also can be integrated into DNA elements designated integrons (Olsson-Liljequist *et al*., 1997). Although integrons by themselves are not mobile, due to their presence in plasmids and transposons, they can be transferred horizontally. For these reasons integrons are a major mechanism for the spread and maintenance of multidrug resistance. (Fluit and Schmitz,1999; Lee *et al*., 2001). Integrons may carry one or more genes in the form of tandem gene cassettes, each of which usually consists of a promoterless open reading frame of about 800 bp. Transcription is initiated by a promoter sequence upstream of the gene cassettes. Each cassette is flanked by conserved sequence, which is recognized by a specialized site-specific recombination enzyme called integrase (*intI*). Because the integron system has the ability to create novel combinations of resistance genes, it may be a dynamic force in the evolution of multidrug resistant (MDR) bacteria. Furthermore, the entire integron element is often contained within other mobile genetic elements such as plasmid

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and transposon, which suggests that entire integron elements including their gene cassettes can spread horizontally through out bacterial populations (Mathai *et al*., 2004).

Integron distributions in uropathogenic *E. coli* have been recently studied in India (Mathai *et al*., 2004), Taiwan (Chang *et al*., 2007), German (Rijavec *et al* ., 2006), Korea (Yu *et al.*, 2003) and USA (Rao *et al.*, 2006). These studies have established a strong association between the presence of integrons and antimicrobial resistance, both MDR and single-drug resistance. However, there is no enough information available on prevalence of integrons classes and their association with drug resistance in uropathogenic *E. coli* in our region.

This study describes the distribution of integron gene cassettes classes in a collection of uropathogenic *E. coli* isolated from children with community acquired UTI.

## Experimental

### Materials and Methods

**Bacteria isolation.** *E. coli* strains were isolated from urine samples of children aged from 1 month to 14 years, referred to Motahary Hospital, Jahrom, Iran. UTI diagnosis was established by the hospital physicians based on clinical symptoms and laboratory investigation. *E. coli* isolates were identified by standard methods (Johnson, 1991). Positive urine cultures were defined by a bacterial growth $>10^5$ colony forming unit/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 15 days and nosocomial infections which were defined as infections noted 48 h after admission or within 4 weeks after a previous discharge.

**Antibiotics susceptibility test.** Susceptibility of all isolates to different antibiotics was determined by the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (Johnson, 1991) with commercial antimicrobial disks (Mast. Co, UK). The antibiotic disks used in this study were: ceforoxime, ceftazidime, norfloxacin, co trimoxazole, tetracycline, chloramphenicol, ampicillin, nalidixic acid, cefixime, gentamicin, nitrofurantoin, ciprofloxacin, amikacin and imipenem. *E. coli* ATCC 25922 was used for quality-control purposes.

**DNA extraction and PCR amplification**. DNA to be amplified was extracted from whole organisms by boiling method (Solberg *et al*., 2006). Bacteria were harvested from 1.5 ml of an overnight Luria-Bertani broth culture (Merck, Germany), suspended in sterile distilled water, and incubated at 95°C for 10 min. Following centrifugation of the lysate, the supernatant was stored at –20°C as a template DNA stock.

Integrons were detected using PCR with degenerate primers designed to hybridize to conserved regions of integron encoded integrase genes *intI1*, *intI2* and *intI3*. The sequences of the primers were as follows: hep35, 5'TGC GGG TYA ARG ATB TKG ATT T 3' and hep36, 5'CAR CAC ATG CGT RTA RAT 3', where B = C or G or T, K = G or T, R = A or G and Y = C or T (White *et al*., 2000). These primers were provided from TIB MOLBIOL Syntheslabor GmbH (Berlin, Germany). PCR amplification was carried out in 50 µl reaction mixtures containing 5 µl DNA template, 50 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs and 2.5 U Taq polymerase. PCR was performed as follows: initial denaturation at 94°C for 5 min followed by 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s and final extension at 72°C for 10 min. 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).

**Detection of integron classes by RFLP-PCR.** The classes of the integrons were determined by analyzing integrase PCR products by restriction fragment length polymorphism (RFLP) (White *et al*., 2001), following digestion using either RsaI or HinfI restriction enzyme (Table I) according to the manufacturer's instruction (MBI, Fermentas, Lithuania).

Table I
Classification of integrase PCR products according to RFLP patterns

| PCR product | Enzyme | No. of fragments | size (s) (bp) |
|---|---|---|---|
| *intI1* | RsaI | 1 | 491 |
| | HinfI | 1 | 491 |
| *intI2* | RsaI | 2 | 334, 157 |
| | HinfI | 2 | 300, 191 |
| *intI3* | RsaI | 3 | 97, 104, 290 |
| | HinfI | 2 | 119, 372 |

**Statistical analysis.** The significance of the results was established using the Fisher's exact test and Yates corrected chi-squared. The level of significance was set at a P value $<0.05$ (Mathai *et al.*, 2004; Rijavec *et al*., 2006).

## Results

**Bacterial strains and antibiotic susceptibility**. Totally 96 strains of *E. coli* were isolated from urine samples of children with community acquired UTI, aged 1 month to 14 years (mean 21.8 ± 26.9 months). The percentage of the strains resistant to the tested

Table II
Antibiotic sensitivity of *E. coli* strains isolated from children with UTI and correlation with integrons

| Antibiotic | Total resistant n (%) | Positive integron n (%) | P value | Positive Integron n (% ) | | P value |
|---|---|---|---|---|---|---|
| | | | | Class I | Class II | |
| Ampicillin | 77 (80.2) | 16 (20.7) | 0.036* | 6 (7.8) | 10 (13) | — — [a] |
| Co-trimoxazole | 73 (76) | 10 (13.6) | 0.34 | 3 (4.1) | 7 (9.6) | 0.2 |
| Tetracycline | 68 (70.8) | 10 (14.7) | 0.36 | 2 (3) | 8 (11.7) | 0.9 |
| Chloramphenicol | 34 (35.4) | 7 (20.5) | 0.57 | 0 (0.0) | 7 (20.6) | — — |
| Nalidixic acid | 24 (25) | 5 (20.8) | 0.17 | 2 (8.3) | 3 (8.8) | 0.6 |
| Cefixim | 19 (19.7) | 5 (26.3) | 0.12 | 2 (10.5) | 3 (15.8) | 0.6 |
| Ceforoxime | 18 (18.7) | 5 (27.7) | 0.17 | 2 (11.1) | 3 (16.5) | 0.67 |
| Gentamycin | 15 (15.6) | 5 (33.3) | 0.02* | 3 (20) | 2 (13.3) | 1 |
| Ceftazidime | 10 (10.4) | 4 (40) | 0.06 | 2 (20) | 2 (20) | 0.77 |
| Ciprofloxicin | 8 (8.3) | 1 (12.5) | 0.23 | 1 (12.5) | 0 (0.0) | 0.35 |
| Norfloxacin | 8 (8.3) | 1 (12.5) | 0.62 | 1 (12.5) | 0 (0.0) | 0.45 |
| Nitrifurantoin | 3 (3.1) | 1 (33.3) | 0.34 | 1 (33.3) | 0 (0.0) | 0.7 |
| Amikacin | 3 (3.1) | 0 (0.0) | — — | 0 (0.0) | 0 (0.0) | — — |
| Imipenem | 0 (0) | 0 (0.0) | — — | 0 (0.0) | 0 (0.0) | — — |

* significant values
[a] because no integron was detected in ampicillin sensitive strains the *P* value could not be determined

antimicrobials is presented in Table II. Among the drugs under the study ampicillin, co-trimoxazol and tetracycline have the least antimicrobial effects. No resistance to imipenem was seen among the strains. Forty six patterns of resistance have been recognized for the *E. coli* strains. These patterns for all isolates are shown in Table III. Seventy seven percent of the isolates were resistant to three or more antibiotics and were designated as multidrug resistant (MDR). Multiple resistances to nalidixic acid, cefixime, ciprofloxacine, ampicillin, nitrofurantoin, chloramphenicol, tetracycline, ceforoxime, ceftazidime, and co-trimoxazole or nalidixic acid, cefixime, ciprofloxacine, ampicillin, gentamicin, tetracycline, norfloxacin, ceforoxime, ceftazidime, and co-trimoxazole were seen in 2.08 percent of the isolates, but no case of multiple drug resistance to all drugs was seen. Only 8.3% of the strains were fully susceptible to all tested antibiotics. The remaining strains were resistant to one or more antibiotics.

**Prevalence of integron among the strains.** Of 96 *E. coli* isolates tested, only 16 strains (16.6%) had the evidence of integron sequences. The incidence of class 1 and class 2 integrons among *E. coli* isolates from urine specimens collected in this study is shown in Table II. Totally, the prevalence of *intI1* and *intI2* among the strains was 6.25% (n = 6) and 10.41% (n = 10), respectively. No *intI3* was detected in the isolates. Resistance to ampicillin (p = 0.036) and gentamicin (p = 0.02) was significantly more common in isolates with integrons compared to those without. Among resistant strains no significant relationship between carrying *intI1* and *intI2* with resistance to antibiotics was observed. As shown in Table III, in the isolates with integrons, there appeared to be a pattern in acquiring resistance, with ampicillin followed by tetracycline and co-trimoxazole.

## Discussion

The level of antibiotic resistances among hospital and community-acquired isolates has steadily increased and has become a major global health problem. Resistance of isolates from urinary tract infections (UTI), which is one of the most frequent infectious diseases and the most common infection in hospitals and extended care institutions, is also changing (Kahlmeter, 2003). In an effort to gain new insight into the emergence of strains that exhibit antimicrobial resistance, 96 UPEC strains isolated from children with community acquired urinary tract infections were characterized with regard to antimicrobial drug resistance and genetic elements involved in DNA mobility.

Among the studied uropathogenic *E. coli* (UPEC) strains from Jahrom, Iran, a high incidence of antibiotic resistances was determined. Even though resistance to tetracycline was high (70.8%), the most prevalent were resistances to ampicillin (80.2%) and co-trimoxazole (76%). Mathai *et al.* (2004) showed in their study high resistances to ampicillin, tetracycline, co trimonazole and sulphonamide among the UPEC strains isolated in Southern India. In another study by Rijavec *et al.* (2006) a high incidence of antibiotic resistances among the UPEC strains from Ljubljana, Slovenia to ampicillin, tetracycline and chloramphenicol

Table III
Distribution of integrons among uropathogenic *E. coli* isolates showing different patterns of drug resistance

| Antibiotic resistance patterns | Integron | | |
|---|---|---|---|
| | Class I | Class II | Total |
| Ap | 2 | 1 | 8 |
| Ts | — — | — — | 6 |
| Na | — — | — — | 1 |
| Ap-Te | — — | — — | 4 |
| Cxm-Ts | — — | — — | 1 |
| Cfm-Ap | — — | — — | 1 |
| Ap-Gm | 1 | — — | 1 |
| Ap-Te-Ts | — — | — — | 13 |
| Ap-C-Te | — — | 2 | 2 |
| Ap-Gm-Ts | 1 | 1 | 1 |
| Na-Te-Ts | — — | — — | 1 |
| Na-Ap-Te | — — | — — | 1 |
| C-Te-Ts | — — | — — | 1 |
| Ap-Gm-Te-Ts | — — | — — | 1 |
| Ap-C-Cxm-Ts | — — | — — | 1 |
| Ap-Ni-Te-Ts | — — | — — | 1 |
| Ap-C-Te-Ts | — — | 2 | 12 |
| Cfm-Ap-Te-Ts | — — | — — | 3 |
| Ap-Ak-Gm-Te-Ts | — — | — — | 1 |
| Na-Ap-C-Te-Cxm | — — | — — | 1 |
| Na-Ap-C-Te-Ts | — — | — — | 2 |
| Na-Cfm-Ap-Te-Ts | — — | — — | 2 |
| Na-C-Te-Cxm-Ts | — — | — — | 1 |
| Cfm-Ap-Te-Cxm-Ts | — — | 1 | 1 |
| Na-Ap-Gm-Te-Ts | — — | — — | 1 |
| Cip-Ap-Gm-Te-Ts | — — | — — | 1 |
| Ap-C-Te-Nor-Cxm-Ts | — — | — — | 2 |
| Ap-C-Te-Nor-Caz-Ts | — — | — — | 1 |
| Na-Ap-Gm-C-Te-Ts | — — | 1 | 1 |
| Na-Cip-Ap-Gm-Te-Ts | — — | — — | 1 |
| Cfm-Ap-Te-Cxm-Caz-Ts | — — | — — | 1 |
| Cfm-Ap-C-Te-Nor-Ts | — — | — — | 1 |
| Cfm-Ap-Ak-Te-Cxm-Caz-Ts | — — | — — | 1 |
| Na-Ap-C-Te-Cxm-Caz-Ts | — — | — — | 1 |
| Na-Cip-Ap-Gm-C-Te-Ts | — — | — — | 1 |
| Na-Ap-Ge-C-Te-Nor-Cxm-Ts | — — | — — | 1 |
| Na-Cfm-Cip-Ap-Gm-C-Te-Ts | — — | — — | 1 |
| Na-Cfm-Ap-C-Te-Cxm-Caz-Ts | — — | 2 | 1 |
| Na-Cip-Ap-Ge-C-Te-Nor-Ts | — — | — — | 1 |
| Na-Cfm-Ap-Ni-Te-Cxm-Caz-Ts | 1 | — — | 1 |
| Na-Cfm-Cip-Ap-Gm-C-Te-Cxm-Ts | — — | — — | 1 |
| Cfm-Ap-Ak-Gm-Te-Nor-Cxm-Caz-Ts | — — | — — | 1 |
| Na-Cfm-Cip-Ap-Ni-C-Te-Cxm-Caz-Ts | — — | — — | 1 |
| Na-Cfm-Cip-Ap-Gm-Te-Nor-Cxm-Caz-Ts1 | 1 | — — | 1 |
| Sensitive | — — | — — | 8 |
| Total | 6 | 10 | 96 |

was determined. High level of resistance to ampicillin (63%), co-trimoxazole (48%) and tetracycline (57.5%) among *E. coli* strins isolated from urine samples has also been documented in Shiraz, Iran (Japoni *et al*., 2008). However, the incidence of resistances to these antibiotics was higher in our UPEC strains compared to these strains. As Jahrom is a small city located in southeast of Shiraz, increasing in anti-

biotic resistance observed in this study could be due to an irrational consumption rate of antibiotics and food from animals that have received antibiotics, transmission of resistant isolates between people, self medication and non-compliance with medication.

No resistance to imipenem was observed in the isolates studied. The same result has been obtained by Adwan *et al.* (2004). They found no imipenem-resistant *E. coli* isolates from UTI. High sensitivity of *E. coli* strains to imipenem has also been reported earlier (Mathai *et al*., 2004; Japoni *et al*., 2008; Tariq *et al*., 2006; Gulsun *et al*., 2005). It seems that this antibiotic can serve as drug of choice for treatment of UTI caused by *E. coli*. However, it should be noted that non limited administration of a drug can gradually lead to rising in antibiotic resistance.

Resistance to nalidixic acid and chloramphenicol 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). 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 in this study which could be explained by low prescription of these antibacterials for urinary tract infections in patients in Jahrom. A high incidence of multidrug-resistant (MDR) strains was also detected among the isolates. About seventy seven percent of the isolates were resistant to three or more tested antibiotics. The level of multidrug resistance among UT1 isolates varies from country to country. It was reported to be 7.1% in 2000 in USA (Sahm *et al*., 2000; Gulsun *et al*., 2005) and 42% of the UPEC isolates in 2006 in Slovenia (Rijavec *et al*., 2006). Such multidrug resistance has serious implications for the empirical therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multidrug resistance plasmids.

Having examined the role of integrons in the prevailing antimicrobial resistance situation in south of Iran we found that 16.6% of the UPEC strains harbored integron sequences. There are only a few studies which have made systematic surveys of integron distribution. One of the first studies was by Sallen *et al.* (1995) who showed integrons in 59% of the isolates belonging to six different species of *Enterobacteriaceae*. Some of these isolates carried multiple integrons. The prevalence of integrons ranging from 22 to 59% has been reported in clinical *E. coli* isolates (Fluit and Schmitz, 1999; Martinez-Freijo *et al.*, 1998). All these data suggest that integrons are common worldwide, especially in *Enterobacteriaceae,* and that they contribute to resistance.

Considering the low prevalence of integrons in our isolates, one may suppose that in these isolates the resistance genes cassettes might be carried on the other transposable elements such as transposons or prophages rather than integrons. In the strains studied integrons were significantly associated with resistance to certain antibiotics including gentamicin and ampicillin (Table II). It has been cited that resistance to gentamicin could be directly related to the presence of resistance genes within the integrons, while the association of the older antibiotic ampicillin with the presence of an integron is likely to be due to genetic linkage between integrons and conjugative plasmids and transposons (White *et al*., 2001).Moreover, our data are in line with earlier suggestions that acquisition of resistance determinants may not be a random process (O'Brien, 2002). Combined resistance to ampicillin followed by tetracycline and co-trimoxazole was the starting point for further resistance development. Initial acquisition of one or two important resistance genes might therefore act as a platform for acquiring more resistance genes.

In conclusion, the present data showed high prevalence of resistance to ampicillin, co trimoxazole and tetracycline among the UPEC strains isolated from children with community-acquired UTI in south of Iran. Imipenem with 100% sensitivity was the most effective antibiotic against these strains. The presence of integrons was significantly associated with resistance to certain antibiotics including gentamicin and ampicillin. However, the low prevalence of integrons among the *E. coli* strains under the study and the MDR patterns, suggests that the antibiotic resistance cassettes in these strains presumably are mostly carried on transposable elements or plasmids rather than integrons.

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