

High Frequency of Class 1 Integrons in *Escherichia coli* Isolated From Patients With Urinary Tract Infections in Yasuj, Iran

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Received 2015 January 14; Revised 2015 April 11; Accepted 2015 May 12.

Abstract

Background: Most urinary tract infections (UTI) are caused by *Escherichia coli*. Integrons have an important role in distributing antibiotic resistance genes among bacteria.

Objectives: The aim of this study was to investigate the presence of class 1, 2 and 3 integrons and their association with antibiotic resistance in *E. coli* isolated from patient with UTI in Yasuj, Iran.

Patients and Methods: In this cross-sectional study a total of 200 *E. coli* were collected from 1820 patients diagnosed with UTI that had been referred to two clinical laboratories between February 2013 and November 2014 in Yasuj city, southwest of Iran. Susceptibility of isolates to 11 different antibiotics was determined by the disk agar diffusion method. multiplex-polymerase chain reaction (PCR) was used for detection of class 1, 2 and 3 integrons. The data were analyzed using the SPSS software (version 16) and the chi-square test. A P value of < 0.05 was considered statistically significant.

Results: The highest rate of resistance was observed toward cephalothin (99%) and amoxicillin (76%) while only two (1%) isolates showed resistance to imipenem. Overall, 79% of isolates were multi drug resistant (MDR). Class 1 and 2 integrons were detected in 104 (52%) and 5 (2.5%) isolates respectively, while none of the isolates were positive for class 3 integrons. A significant association was observed between the presence of integrons and resistance to co-trimoxazole, nalidixic acid, ciprofloxacin, amoxicillin, ceftazidime and tetracycline ($P < 0.05$).

Conclusions: High MDR isolates of *E. coli* were observed in this study. The significant association between class 1 integrons and resistance to ciprofloxacin, nalidixic acid, co-trimoxazole, amoxicillin, ceftazidime and tetracycline showed that class 1 integrons have an important role in resistance to these antibiotics in this region.

Keywords: Integrons, Urinary Tract Infections, Multidrug Resistant, *Escherichia coli*

1. Background

Urinary tract infection (UTI) is one of the most prevalent infections in human beings (1). Community acquired UTI and nosocomial infection are two types of urinary tract infections (2). Most of the cases of UTI are commonly caused by gram negative bacteria; *Escherichia coli* is responsible for 80% of infections (3) followed by *Klebsiella pneumonia*, *Acinetobacter* spp and *Enterobacter* spp (4). The Uropathogenic *Escherichia coli* (UPEC) strains are the most important causes of UTIs (5, 6). However, the gram-positive bacteria, *Staphylococcus saprophyticus*, can cause UTI in young females (7). Increasing resistance to antibiotics by UTI-causing organisms is a serious public health problem (8, 9). High levels of resistance to antibiotic agents that are frequently used to treat UTI infections have been reported in UTI-causing

E. coli (10, 11). Horizontal gene transfer (HGT) has an important role in spreading resistance genes among bacteria through mobile genetic elements such as, plasmids, transposons and integrons (12). Integrons are genetic elements that are capable of integration of resistance gene cassettes in their structure and subsequently denote resistance phenotype to their bacterial host (13). Integrons can be located within transposons or conjugated plasmids and transfer along with them, facilitating the dissemination of antibiotic resistance genes between bacteria (14). Structurally, integrons consist of two 5' conserved segments (5'-CS) and 3' conserved segments (3'-CS) that flank the central variable region or gene cassettes, which encode antibiotic resistance traits (15). The 5'-CS of integrons encode the integrase

(*Int1*) gene, the recombination site (*attI*) and the common promoter (Pc), while the 3'-CS harbors the *qacE Δ1* gene and *sulI* gene that mediate resistance to certain detergents and sulphonamides, respectively (16). In addition to encoding resistance genes by gene cassettes, they include a recombination site (*attC*). Recombination between the *attI* and *attC* sites leads to insertion of the gene cassette downstream of a resident promoter, which mediated by the *intI* gene (17). Based on the homology of the *intI* gene, four classes (1-4) of integrons have been identified (18); class 1 integrons are the most prevalent followed by class 2 integrons (19). More than 194 gene cassettes encode antibiotic resistance to aminoglycoside, β-lactams, chloramphenicol, quinolones and trimethoprim (20). One or more gene cassette can be incorporated in integrons, however usually less than five cassettes with numerous combinations are carried by isolated bacteria (20-22). Hence, the presence of integrons among bacteria is associated with multiple drug resistance (MDR), especially in enteric bacteria such as *E. coli* (23). The MDR enterobacteriaceae serve as a major public health problem and a strong association between MDR and the presence of integrons has been shown in Enterobacteriaceae, independent of species or strain origin (24). Many studies have investigated the presence of integrons in *E. coli* isolated from patient with UTI, and they reported a significant association between antimicrobial resistance and existence of integron (23-28). Pattern of resistance to antibiotics change continuously in UTI-causing organisms, therefore appropriate information about local and national antimicrobial resistance will be needed for empirical therapy of UTI (28).

2. Objectives

Considering the importance of regional and local information about antibiotic resistance pattern among *E. coli* isolated from patients with UTIs, and the association between integrons and MDR among gram negative bacteria, the aim of this study was to determine the pattern of antibiotic resistance to commonly used antimicrobial agents, and to investigate the presence of class 1, 2 and 3 integrons and their association with antibiotic resistance in *E. coli* isolated from patient with UTIs in Yasuj, Iran.

3. Materials and Methods

3.1. Sample Collection and Antimicrobial Susceptibility Test

In this cross-sectional study, midstream urine samples of 1820 patients diagnosed with UTIs were collected in sterile universal containers from February 2013 to November 2014.

All patients had attended two clinical laboratories in Yasuj city, southwest Iran. Exclusion criteria were having an indwelling urinary catheter, being pregnant, having genitourinary abnormalities and antibiotics therapy within the previous two weeks. Urine samples were inoculated on MacConkey agar and blood agar plates using calibrated

loops (0.001 mL) and incubated at 37°C for 18 to 24 hours. The number of colony forming units (cfu) was counted. A growth of > 10⁵ colony forming units/mL of one type of organism was considered as significant bacteriuria. The isolates were identified to the species level using conventional biochemical tests, such as indole production, methyl red, Voges-Proskauer, Simmons' citrate, hydrogen sulfide, and urea hydrolysis (29). Finally 200 isolates of *E. coli* were identified. All of the isolates were stored at -20°C in trypticase soy broth (Mast Group Ltd. UK) containing 15% glycerol (29).

Disks containing antibiotics (Himedia, India) were used to determine the susceptibility of *E. coli* isolates to the following antibiotics, according to the clinical and laboratory standards institute (CLSI) guidelines (30): amoxicillin (25 µg), gentamycin (10 µg), amikacin (30 µg), co-trimoxazole (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), imipenem (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg). *Escherichia coli* ATCC 25922 was used as a quality control strain. This study was approved by the ethical committee of Yasuj University of Medical Sciences (ethical code number: 20.11.91-91011020).

3.2. Multiplex-Polymerase Chain Reaction for Detection of Class 1, 2 and 3 Integrons

Genomic DNA was extracted according to the method of Yu et al. (23) with some modifications. Briefly, three to five colonies of overnight culture of *E. coli* on brain heart infusion agar was suspended in 300 µL of sterile distilled water and boiled at 95°C for ten minutes then cell debris was removed by centrifugation for ten minutes at 13000 rpm, and 200 µL of the supernatant was stored at -20°C for DNA amplification.

The *intI1*, *intI2* and *intI3* genes were amplified by a multiplex-polymerase chain reaction (PCR) method using primers described by Goldstein et al. (8) (Table 1). The PCR amplification was performed in a total volume of 25 µL containing 12.5 µL of master mix (Cinnagen, Iran), 25 pmol of each primer and 3 µL of DNA template. The PCR conditions are presented in Table 2. Expected amplified products, 280 bp for integron class 1, 233 bp for integron class 2 and 600 bp for integron class 3 were separated by electrophoresis on 1% agarose gel containing 0.5 µg/mL ethidium bromide and photographed under UV illumination.

3.3. Statistical Analysis

The SPSS software (version 16, Chicago, IL, USA) was used for statistical analysis. The association between presence of integrons and antibiotic resistance was determined by χ^2 or Fisher's exact test. A P value of < 0.05 was considered statistically significant.

4. Results

Among the 200 UTI *E. coli* strains, 144 (72%) strains were isolated from females and 56 (28%) strains were isolated from males. High frequencies of resistance were observed

toward cephalothin (99%) and amoxicillin (76%) while only two (1%) isolates showed resistance to imipenem. The *E. coli* isolates were also resistant to co-trimoxazole (62%), tetracycline (50%), nalidixic acid (48.5%), ceftazidime (40.5%), ciprofloxacin (29%), gentamicin (15.5%), chloramphenicol (13%) and amikacin (3%). Of the 200 tested *E. coli* isolates, 158 (79%) were MDR and were resistant to three or more antibiotics. None of the isolates were fully susceptible or resistant to all of the tested antibiotics.

Sixty-six antibiotic resistance patterns were observed in *E. coli* isolates, yet 30 resistance phenotypes were presented by only one isolate. Resistance to cephalothin was the most common phenotype, which was detected in 27 isolates. The second resistance phenotype was towards 13 isolates, including resistance to cephalothin, amoxicillin, co-trimoxazole and tetracycline.

Class 1 and 2 integrons were detected in 104 (52%) and 5 (2.5%) isolates, respectively, while none of isolates were positive for class 3 integrons. Two isolates had both class 1 and 2 integrons. Ninety-three of the isolates (46.5%) did not contain any classes of integrons. Among the 158 MDR *E. coli*, 101 (63.9%) harbored class 1 or class 2 integrons yet only 5 (11.9%) of the 42 non-MDR isolates had integrons.

The carriage of class 1 integron was found to be significantly higher in co-trimoxazole ($P < 0.001$), nalidixic acid ($P < 0.001$), ciprofloxacin ($P < 0.015$), amoxicillin ($P < 0.001$), ceftazidime ($P < 0.003$) and tetracycline ($P < 0.012$) resistant isolates (Table 3). There was no significant association between resistance to chloramphenicol, amikacin and gentamicin with the presence of class 1 integrons.

Table 1. Sequences of Primers Used for Detection of *intl-1*, *intl-2* and *intl-3* Genes in *Escherichia coli* Isolates

Gene Type/Primer	Sequence (5' → 3')	Product Size, bp	References
intl-1		280	(8)
F:	CCTCCCGCAGATGATC		
R:	TCCAGGCATCGTCAGGC		
intl-2		233	(8)
F:	TTATTGCTGGGATTAGGC		
R:	ACGGCTACCCCTCTGTTATC		
intl-3		600	(8)
F:	AGTGGGTGGCGAATGAGTG		
R:	TGTCTGTATCGGCAGGTG		

Abbreviations: F, forward; R, reverse.

Table 2. Multiplex Polymerase Chain Reaction Conditions for Detection of Class 1, 2 and 3 Integrons Among 200 *Escherichia coli* Isolates

	Temperature, °C	Time, s
First Denaturation	94	240
Extension Final	72	600
Cycles: 30		
Denaturation	94	50
Annealing	55	30
Extension	72	60

Table 3. The Correlation Between Presence of Integrons and Antibiotic Resistance Among 200 *Escherichia coli* Isolated From Patients With Urinary Tract Infections

Antibiotics	Integron Class 1 Positive ^a	Integron Class 2 Positive ^a	Total Resistance	Association With Class 1 Integrons (P Value)
Co-trimoxazole	88 (44)	4 (2)	124	< 0.001 ^b
Chloramphenicol	19 (9.5)	0	26	< 0.44
Tetracycline	55 (27.5)	4 (2)	100	< 0.012 ^b
Amikacin	2 (1)	0	6	< 0.28
Gentamicin	21 (10.5)	2 (1)	31	< 0.09
Ciprofloxacin	38 (19)	3 (1.5)	58	< 0.015 ^b
Nalidixic acid	63 (31.5)	4 (2)	97	< 0.001 ^b
Amoxicillin	93 (46.5)	5 (2.5)	152	< 0.001 ^b
Ceftazidime	52 (26)	5 (2.5)	81	< 0.003 ^b
Cephalothin	104 (52)	5 (2.5)	198	NA ^c
Imipenem	0	0	2	NA ^d

Abbreviation: NA, not available.

^aValues are expressed as No. (%).

^bP values that indicate significance.

^cOnly two sensitive isolates.

^dOnly 2 resistant isolates.

5. Discussion

Consistent with previous reports, in this study class 1 integrons were the most prevalent compared to the other tested integrons. In accordance with our study, Chang et al. from Taiwan and Li et al. from China detected class 1 integrons in 64% and 66.5% of *E. coli* isolated from different clinical specimens and blood stream infections, respectively (31, 32). Many reports have shown the prevalence rate of integrons to be between 6.25% and 54.6% of clinical isolates (33-38). There have been various studies from different cities of Iran: Fallah et al. from Tehran detected class 1 integrons in 50.3% of isolates (37) while Rezaee et al. from Tabriz, and Japoni et al. and Farshad et al. from Shiraz identified this class of integrons in 22.05%, 33.34% and 6.25% of *E. coli*, respectively (33-35). Farshad et al. detected integrons in only 6.25% of *E. coli* isolated from children with UTI and suggested that antibiotic resistance cassettes may be carried on transposable elements or other plasmids rather than integrons. Class 2 integrons were detected in 2.5% of isolates in this study, which is lower than that reported by other studies, such as Fallah et al. (12.5%), Farshad et al. (10.41%), Rezaee et al. (5.08%), Cao et al. (5.88%) and (6.7%) Japoni et al. (33-35, 37, 39). Similar to previous studies we couldn't find class 3 integrons in the present study (31-39). Antibiotic resistance encoding gene cassettes for fluoroquinolones, β -lactams, aminoglycosides, trimethoprim and chloramphenicol have been detected in integrons (20). A significant association between presence of class 1 integrons and resistance to co-trimoxazole, nalidixic acid, ciprofloxacin, amoxicillin, ceftazidime and tetracycline was shown in this study. In accordance with our study, a significant association between presence of class 1 integrons and resistance to ciprofloxacin, nalidixic acid and co-trimoxazole was reported by Japoni et al, to co-trimoxazole, nalidixic acid and ceftazidime by Fallah et al. and to nalidixic acid and tetracycline by Mathai et al. (27, 35, 37). While in many studies resistance to other antibiotics such as gentamicin, amikacin, cephalothin (35) and chloramphenicol (34) were correlated with class 1 integrons. In spite of many resistance gene cassettes including *aadA*, *aadB*, *aadA7*, *aadA4* and *aacAI*, which confer resistance to aminoglycosides like amikacin and gentamicin, in our study this association was not significant. This may be due to the low resistance rate to amikacin (2.5%), or other resistance mechanisms (mutation in the rRNA gene, efflux and permeability mechanisms) to aminoglycosides rather than integrons by bacteria in this study. Since most of the isolates (99%) were resistant to cephalothin and only 1% were resistant to imipenem, no significant association was found between presence of integrons and resistance to these antibiotics, similar to many previous studies (33, 34). In this study, 79% of isolates were MDR, which is approximately similar to other reports from Iran that showed 77% and 84.2% MDR in isolated *E. coli* (33-37), while in the USA and Slovenia this was 7.1% and 42%, respectively (39, 40). Empirical therapy for MDR isolates is a major concern; hence a periodical local study will be needed to assess the antibi-

otic susceptibility pattern. In the present study a high resistance rate was seen towards cephalothin (99%), which is higher than other studies that reported 77.8%, 60% and 40% (34, 35, 37). Extreme sensitivity of *E. coli* to imipenem was observed in this study. Previous studies by Tariq et al. Japoni et al. Farshad et al. and Rezaee et al. showed similar results (33-35, 41) while, Fallah et al. reported that 27.1% of their isolates were sensitive to imipenem (37). Although this antibiotic may be used as a drug of choice for treatment of UTI caused by *E. coli*, yet it is only recommended for complicated urinary tract infections. Resistance to ciprofloxacin and nalidixic acid was 29% and 48.5% in this study, which was lower than the studies of Fallah et al. and Rezaee et al. (34, 37), yet higher than other studies (35, 42, 43). These two antibiotics are among the drugs of choice for treatment of UTI, hence increased use of these antibiotics without knowledge about the pattern of antibiogram may gradually lead to a rise in antibiotic resistance. Sixty-two percent of the isolates were resistant to co-trimoxazole, which was similar to the study of Fallah et al. (67.7%) but higher than the study of Japoni et al. and lower than Rezaee et al. and Farshad et al. (33-35, 37).

5.1. Conclusions

A large number of MDR isolates of *E. coli* were observed in this study, among which 62.6% had class 1 integrons. The significant association between class 1 integrons and resistance to ciprofloxacin, nalidixic acid, co-trimoxazole, amoxicillin, ceftazidime and tetracycline suggests that class 1 integrons have an important role in resistance to these antibiotics. We suggest that further research should be done on the association between antibiotic resistance and presence of integrons, integrase genes and gene cassettes.

Acknowledgments

We are grateful to Yasuj University of Medical Sciences for their financial support.

Footnotes

Authors' Contribution: Study concept and design: Seyed Sajjad Khoramrooz; acquisition of data: Seyed Ali Asghar Malek Hosseini; analysis and interpretation of data: Mehdi Mirzaei and Seyed Abdolmajid Khosarvani; drafting of the manuscript: Seyed Sajjad Khoramrooz, Mohammad Emaneini and Farzaneh Gharibpour; critical revision of the manuscript for important intellectual content: Mohammad Emaneini, Seyed Sajjad Khoramrooz and Farzaneh Gharibpour; statistical analysis: Mohammad Zoladl; administrative, technical and material support: Mahboubeh Yazdanpanah, Seyed Ali Asghar Malek Hosseini and Najmeh Parhizgari; study supervision: Seyed Sajjad Khoramrooz and Asghar Sharifi.

Funding/Support: This research was supported by the deputy of research and technology, Yasuj University of Medical Sciences.

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