Journal of Acute Disease

Original Article

doi: 10.4103/2221-6189.259110 jadweb.org

Integron frequency of *Escherichia coli* strains from patients with urinary tract infection in Southwest of Iran

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ARTICLE INFO

Article history:
Received 28 January 2019
Revision 29 March 2019
Accepted 28 April 2019
Available online 27 May 2019

Keywords:
Escherichia coli
Urinary tract infection
Integrons
IntI1

ABSTRACT

Objective: To investigate the frequency of integrase genes *intI1*, *intI2* and *intI3* of *Escherichia coli* strains, and their association with resistance to routinely used antibiotics.

Methods: A total of 120 *Escherichia coli* strains were collected from patients with urinary tract infection in Ahvaz, Southwest of Iran. Antibiotic susceptibility testing was performed. The presence of *intI1*, *intI2*, and *intI3* genes was determined by polymerase chain reaction.

Results: Antibiotic susceptibility testing disclosed the highest resistance rate to ampicillin (91.7%) followed by trimethoprim/sulfamethoxazole (65.8%), and ceftazidime (56.7%). The imipenem susceptibility rate was 91.7%. *IntI1* and *intI2* were identified in 74 (61.6%) and 8 (6.6%) of *Escherichia coli* strains, respectively, but *intI3* was not found in any isolates. The presence of integrons was significantly associated with resistance to ampicillin, trimethoprim/sulfamethoxazole, ceftazidime, and ciprofloxacin antibiotics (P<0.05).

Conclusions: The high resistant *Escherichia coli* isolates harboring class 1 integrons (*intI1*) were detected in patients with urinary tract infection in our region. Therefore, preventive strategies are necessary to restrict further dissemination of resistant strains.

1. Introduction

Due to frequent relapses and complications, urinary tract infections (UTIs) are serious health problems around the world[1]. It is estimated that UTIs accounts for more than 12% of all nosocomial infections[2]. In the United States, over 7 million medical visits were recorded due to UTIs, of which 100 000 patients required hospitalization. The total expenses related to the treatment are estimated at around 1.6 billion USD per year[3]. So, the proper treatment of UTIs is very important to reduce the incidence rate and costs.

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How to cite this article: Alkhudhairy MK, Saki M, Seyed-Mohammadi S, Jomehzadeh N, Khoshnood S, Moradzadeh M, et al. Integron frequency of *Escherichia coli* strains from patients with urinary tract infection in Southwest of Iran. J Acute Dis 2019; 8(3): 113-117.

The main etiological factors of UTI are bacteria. Among the bacteria, bacilli of the Enterobacteriaceae family, especially *Escherichia coli* (*E. coli*) strains are still the most common cause of infection[4]. Genetic components, including transposons, integrons, and plasmids of Gram-negative bacteria, play an important role in the emergence of antibiotic resistance[5,6]. Integrons are genetic elements capable of integrating or transporting specific gene cassettes that encode antibiotic resistance determinants[7]. Structurally, all integrons are composed of three main components: a tyrosine recombinase gene or integrase (*intI*), an integron-associated recombination site (*attI*), and a promoter site (*Pc*)[5,7]. So far, 5 diverse types of mobile integrons have been identified, all of which are associated with antibiotic resistance[7].

Several reports have shown the importance of integrons in creating antibiotic resistance of *E. coli* strains isolated from UTIs[8,9]. However, the prevalence of integrons in the *E. coli* strains isolated from UTI patients has not been reported from Ahvaz, southwest of Iran. In that sense, the estimation of the local distribution of integrons and their association with resistance to antibiotics can provide basis for further studies on the causes of antibiotic resistance in multi-drug resistant (MDR) *E. coli* strains in order to prevent the spread by appropriate policies.

The aims of this study were to estimate the prevalence of class 1, class 2, and class 3 integrons and their association with resistance to routinely used antibiotics in *E. coli* strains isolated from patients with UTI in Ahvaz, southwest of Iran.

2. Materials and methods

2.1. Ethical consideration

This study protocol was approved by the local ethics committee of the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, and the study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients before participation.

2.2. Sample collection and isolation of E. coli

From April 2017 to May 2018, a total of 380 urine specimens were obtained from patients with UTI referred to the Golestan Hospital, a major referral center in Ahvaz city, affiliated to the Ahvaz Jundishapur University of Medical Sciences, southwest of Iran. Urine samples were collected by midstream clean-catch method. All urine samples were cultured on blood agar, MacConkey agar, and eosin-methylene blue agar (Merck, Darmstadt, Germany) with a standard wire loop and incubated at 37 ℃ overnight. A pure bacterium growth (colony forming units/mL equal or more than 10^5) was considered as definitive UTI. Identification of the isolates was performed according to standard microbiologic methods such as Gram-stain, triple sugar iron utilization, citrate utilization, urea test, indole test, and methyl red-Voges Proskauer tests[10]. The strains that confirmed as *E. coli* were transferred to microbiology laboratory of the Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences within 2 h and stored in trypticase soy broth containing 30% glycerol at -70 ℃ for long preservation and further analysis.

2.3. Antibiotic susceptibility testing (AST)

The antibiogram of all isolates was done against nine routinely used antibiotics by standard disk diffusion method on Mueller-Hinton agar medium (Merck, Darmstadt, Germany) according to the Clinical and Laboratory Standards Institute procedures, 2016[11]. The antibiotics were ciprofloxacin (5 μg), ceftazidime (30 μg), gentamicin (10 μg), cefoxitin (30 μg), cefepime (30 μg), cefotaxime (30 μg), imipenem (10 μg), ampicillin (10 μg), and trimethoprim/sulfamethoxazole (25 μg) (MAST, Berkshire, UK). The MDR isolates were determined according to resistance to three or more of antimicrobial classes[12]. *E. coli* ATCC 25922 was used for the quality control of AST assay.

2.4. DNA extraction

Extraction of DNA from all isolates was performed by the boiling method. Briefly, one pure colony of an overnight culture of *E. coli* on nutrient agar (Merck, Darmstadt, Germany) was suspended in 500 μL of sterile Tris-EDTA buffer and boiled at 95 ℃ for 10 min, then was centrifuged for 10 min at 14 000 rpm. Finally, 300 μL of the supernatant was stored at -20 ℃ for polymerase chain reaction (PCR). The concentration and purity of DNA were determined using a NanoDrop spectrophotometer (Thermo Scientific, USA) and agarose gel electrophoresis, respectively.

2.5. Multiplex PCR for detection of intI1, intI2 and intI3 genes

The occurrence of *intI1*, *intI2*, and *intI3* genes was screened in all isolates by the multiplex PCR method using previously described primers(Table 1)[13]. The PCR assay was performed in the Eppendorf thermocycler 5530 (Roche, Mannheim, Germany) in the following condition: an initial denaturation at 94 ℃ for 5 min, followed by 35 cycles at 94 ℃ for 30 s, annealing at 60 ℃ for 1 min, extension at 72 ℃ for 45 s and a final extension at 72 ℃ for 5 min, respectively. PCR reaction was carried out in a final volume of 25 μL including 12.5 μL of master mix (Sinaclon, Tehran, Iran), 1 μL of forward and reverse primers (10 pmol), 5.5 μL of deionized water, and 1 μL of DNA sample. The expected amplicons were separated by electrophoresis (70 V, 45 min) on 1.0% agarose gel prepared in 1×TAE (Tris/Acetate/EDTA) buffer containing 0.5 μg/mL ethidium bromide (Sinaclon, Tehran, Iran) and screened using the gel documentation system (ProteinSimple, San Jose, CA, USA). In every PCR run, the positive and negative controls were used. The PCR products were confirmed by direct sequencing.

Table 1. Sequences of primers used in this study[13].

Primers	Sequence (5' to 3')	Product size (bp)
intI1	F-GGTCAAGGATCTGGATTTCG	436
	R-ACATGCGTGTAAATCATCGTC	
intI2	F-CACGGATATGCGACAAAAAGG	788
	R-TGTAGCAAACGAGTGACGAAATG	
intI3	F-AGTGGGTGGCGAATGAGTG	600
	R-TGTTCTTGTATCGGCAGGTG	

2.6. Statistical analysis

All data were analyzed with SPSS™ software, version 22.0 (IBM Corporation, Armonk, NY, USA). The results were shown as descriptive statistics. The association of integrons with antibiotic resistance was determined by *Chi*-square and Fisher's exact test. The statistically significant association was considered based on a *P*-value <0.05.

3. Results

3.1. Bacterial isolates

In this study, a total of 120 *E. coli* strains were collected from urine samples, showing an overall prevalence of 31.6% (120/380) of *E. coli* in UTIs. Among the 120 *E. coli* isolates, 36 (30%) and 84 (70%) strains were collected from males and females, respectively. The age of the patients was (10-86) years (average 46 years).

3.2. Antibiotic resistance patterns

According to the results of AST, the highest antibiotic resistance was observed toward amoxicillin (91.7%) and trimethoprim/sulfamethoxazole (65.8%), followed by ceftazidime (65.0%), while the lowest resistance was observed against imipenem (7.5%). Ten isolates were completely susceptible to these nine antibiotics (Table 2).

Table 2. Antibiotic sensitivities of *E. coli* isolates from patients with UTIs [*n* (%)].

Antibiotics	Resistant	Intermediate	Susceptible
Ciprofloxacin	77 (64.2)	3 (2.5)	40 (33.3)
Ceftazidime	78 (65.0)	4 (3.3)	38 (31.7)
Gentamicin	71 (59.2)	7 (5.8)	42 (35.0)
Cefoxitin	76 (63.3)	4 (3.3)	40 (33.3)
Cefepime	60 (50.0)	6 (5.0)	54 (45.0)
Cefotaxime	67 (55.8)	5 (4.2)	48 (40.0)
Imipenem	9 (7.5)	1 (0.8)	110 (91.7)
Ampicillin	110 (91.7)	2 (1.7)	8 (6.7)
Trimethoprim/ sulfamethoxazole	79 (65.8)	0	41 (34.2)

Table 3. Multidrug-resistant profiles of *E. coli* isolates.

Multidrugs	*n* (%)
CIP-CAZ-GEN-FOX-FEP-CTX-AMP-SXT	25 (25.8)
CIP-CAZ-GEN-FEP-AMP-SXT	10 (10.3)
CIP-CAZ-FOX-CTX-AMP-SXT	12 (12.4)
CIP-CAZ-GEN-AMP-SXT	1 (1.0)
CIP-GEN-FOX-CTX-AMP	10 (10.3)
CIP-CAZ-GEN-FOX-AMP	10 (10.3)
CAZ-AMP-SXT	1 (1.0)
CIP-AMP-IMI-SXT	9 (9.3)
CAZ-GEN-FOX-FEP-AMP-SXT	15 (15.5)
CAZ-FOX-AMP-SXT	4 (4.1)

CIP: ciprofloxacin; CAZ: ceftazidime; GEN: gentamicin; FOX: cefoxitin; FEP: cefepime; CTX: cefotaxime; IMI: imipenem; AMP: ampicillin; SXT: trimethoprim/sulfamethoxazole.

3.3. MDR profiles

Among the 120 *E. coli* isolates, 97 (80.8%) were MDR with 10 dissimilar profiles (Table 3). The most prevalent resistance profile was the CIP-CAZ-GEN-FOX-FEP-CTX-AMP-SXT with the rate of 25.8 %.

3.4. Distribution of intI1, intI2 and intI3 genes

Among the 120 *E. coli* strains, 78 (65.0%) isolates had the integrons genes. The *intI1* and *intI2* genes were detected in 74 (61.7%) and 8 (6.7%) strains respectively, however, the *intI3* gene was not identified in any of the isolates. Simultaneous occurrence of *intI1* and *intI2* was detected in 4 (3.3%) isolates. Also, the presence of integron genes was significantly associated with the higher resistance to ampicillin, trimethoprim/sulfamethoxazole, ceftazidime, and ciprofloxacin antibiotics ($P<0.05$) (Table 4). The presence of integrons did not have a significant association with resistance to gentamicin, cefoxitin, cefepime, cefotaxime and imipenem antibiotics.

Table 4. Antibiotic resistance pattern of *E. coli* isolates according to integron positivity [*n* (%)].

Antibiotics	Integron positive (*n*=78)		Integron negative (*n*=42)		*P* value
	Resistant	Susceptible	Resistant	Susceptible	
Ciprofloxacin	63 (80.8)	15 (19.2)	14 (33.3)	25 (59.5)	< 0.001
Ceftazidime	60 (76.9)	17 (21.8)	18 (42.9)	21 (50.0)	< 0.001
Gentamicin	45 (57.7)	30 (38.5)	26 (61.9)	12 (28.6)	NS
Cefoxitin	48 (61.5)	28 (35.9)	28 (66.7)	12 (28.6)	NS
Cefepime	40 (51.3)	34 (43.7)	20 (47.6)	20 (47.6)	NS
Cefotaxime	42 (53.8)	33 (42.3)	25 (59.5)	15 (35.7)	NS
Imipenem	6 (7.7)	72 (92.3)	3 (7.1)	38 (90.5)	NS
Ampicillin	78 (100)	0 (0)	32 (76.2)	8 (19.0)	< 0.001
Trimethoprim/ sulfamethoxazole	65 (83.3)	13 (16.7)	14 (33.3)	28 (66.7)	< 0.001

NS: not significant.

4. Discussion

In more than 95% of the UTI cases, only one microbial agent is diagnosed as infection, of which about 70%-95% are *E. coli* species[14,15]. Today, the antibiotic resistance among Gram-negative bacteria, including *E. coli*, has become a serious problem. The integrons are known as the source of transferring drug resistance genes in bacterial populations[16,17].

Previous studies have confirmed that the presence of these elements, especially class 1 integron, can be considered as evidence for a multi-drug resistance phenotype in *E. coli* isolates[18,19]. Therefore, in addition to providing basic epidemiologic information, the antibiotic resistance patterns and the prevalence of integron genes in UTI *E. coli* isolates can be used by general practitioners and healthcare managers to monitor and plan for effective empiric treatments against MDR isolates.

The data from the present study showed a high rate of antibiotic resistance among UTI *E. coli* isolates. More than ninety percent of isolates were resistant to at least one antibiotic. In agreement with the findings of previous studies carried out at different countries in the world, in this study, the highest resistance rate of *E. coli* isolates was detected against ampicillin and trimethoprim/sulfamethoxazole[20,21]. Furthermore, *E. coli* isolates showed the highest sensitivity to imipenem which was in line with results of the previous studies in Iran and other countries[19,22]. However, contrary to the results of our study, in most previous studies resistance to imipenem in *E. coli* isolated from UTIs has been reported less than 5%[19].

In this study, among the integron positive *E. coli* strains, the resistance rate against cephalosporins varied from 51.3% to 76.9%. Meanwhile, cefepime with 43.7% of the susceptibility rate, was the most effective cephalosporin against integron positive *E. coli* isolates). Sixty-five percent of the isolates were resistant to ceftazidime, while Khoramrooz *et al*. reported the resistance rate of 40.5% for their isolates[23]. Due to the relatively high resistance of *E. coli* isolates to third-generation cephalosporins in this study, bacterial screening for the production of extended-spectrum beta-lactamases should be considered in subsequent studies. In this study, high resistance was observed toward ciprofloxacin. Despite the fact that the published results in different countries have shown the effectiveness of fluoroquinolones in the treatment of UTIs[24,25], according to the results of AST and the high resistance to ciprofloxacin in our study, the administration of these antibiotics in empirical treatment of UTIs should be revised in our region. In the current study, the rate of multi-drug resistance among *E. coli* isolates was much higher than the study in Saudi Arabia[20], which could be attributed to the lack of precise control programs for the prescribing of antibiotics.

Various studies have been done in our country regarding the incidence of integrons in *E. coli* strains with clinical source[18,19,23]. In line with past reports, integrons class 1 were more common in comparison to the other two classes in our study. Similar to our results, Yekani *et al.* from Iran detected class 1 integrons in 63.6% of uropathogenic *E. coli* isolates[19]. In another Iranian research by Fallah *et al.*, 50.3% of *E. coli* isolates were positive for class 1 integrons[26]. In this study, the class 2 integrons were detected in 8 (6.7%) isolates as compared to 4.5% to 12.5% prevalence of class 2 integrons reported from other regions of Iran[18,19,23,26]. Meanwhile, similar to most previous studies from Iran and other countries, none of the isolates had class 3 integrons[18,19,23,27]. So far, the class 3 integrons have only been reported in diarrheagenic *E. coli* isolates in southwestern Iran[13].

Association of integrons with resistance to aminoglycosides, quinolones, chloramphenicol, trimethoprim, tetracycline, and β-lactam antibiotics has been proven in previous studies[23,28]. In this study, a remarkable correlation was revealed between the occurrence of integrons and the resistance to ceftazidime (P<0.001), ciprofloxacin (P<0.001), ampicillin (P<0.001), and trimethoprim/sulfamethoxazole (P<0.001). In accordance with a study conducted by Khoramrooz *et al*. in Iran, a statistically significant link between the integrons and resistance to ciprofloxacin, ceftazidime, and cotrimoxazole was found[23]. However, contrary to our study, Fallah *et al*. found that the integrons had a significant association with the resistance to imipenem[26].

In summary, in our region, the majority of *E. coli* isolates causing UTIs are MDR. The abundant prevalence of integrons class 1 and 2 among the *E. coli* isolates may be contributing to the resistance to common antibiotics. Therefore, the periodical and regular determination of the drug resistance patterns of *E. coli* isolate is necessary in order to reduce the infection rate with MDR isolates and to prevent the spread of these strains in our region.

Conflict of interest statement

The authors report no conflict of interest.

Acknowledgments

We would like to thank the staff of the Microbiology Department of the Golestan Hospital of Ahvaz city for their valuable assistance.

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