Association of integrons with multidrug-resistant isolates among phylogenic groups of uropathogenic *Escherichia coli*

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Abstract

The aims of this study were to investigate the antibiotics susceptibility, multidrug-resistant (MDR) frequency and the association of integrons with MDR among phylogenic groups of uropathogenic *E. coli* (UPEC). In total, 176 non-duplicated UPEC isolates were collected from urinary tract infections (UTIs) specimens. The disk diffusion method was performed for determination of antibiotic susceptibility patterns. Phylogenetic grouping and the presence of integron-associated genes (*int)* were detected by the PCR technique. A high frequency of resistance was observed to cotrimoxazole (96.9%), ampicillin (85%), trimethoprim (80.1%) and cefazolin (79.6%); and 140 isolates (79.5%) were MDR. Carbapenems and fosfomycin were the most effective antibiotics. The majority of isolates (60.8%) belonged to the phylogenic group B2. Integrons were detected in 135 (76.7%) of isolates and, class I was the most common (63.6%) class. MDR isolates were found to be significantly associated with class I integrons. These isolates were found to be closely associated with the phylogenic group D (82%), however, the presence of class I integrons was higher among MDR isolates of the phylogroup B1. This pattern is believed to be due to other mechanisms such as the overexpression of the efflux pumps. Our findings show a significant correlation between MDR and the presence of class I integron. We conclude that class 1 integron plays an important role in the development of MDR UPEC, especially among the phylogroup B1.

Introduction

Urinary tract infections (UTIs) are the most common bacterial infections that result in a significant number of physician visits and hospital admission.[1] Increasing prevalence of drug-resistant bacteria causing UTIs is a considerable public health challenge.[2] *Escherichia coli* are the most common etiologic agent of the community and hospital-acquired UTIs.[3,4] Many studies have demonstrated the increasing antibiotics resistant *E. coli* causing UTIs that complicates the management of infections due to this organism. The high frequency of resistance to some of β-lactams, quinolones, aminoglycosides and trimethoprim-sulfamethoxazole were described among Uropathogenic *E. coli* (UPEC).[5,6] Multidrug resistance (MDR) occurs as a result of the current treatment of urinary tract infections.[7] MDR isolates acquire and spread horizontal genetic elements of antibiotics resistance by the integron.[8] Integrons are genetic determinants that are competent of capture, intercommunicate and express genes within gene cassettes and confer resistance to antibiotics.[9,10] These genetic elements have strong promoters and are capable of promoting direct transcriptions of captured genes.[11] Integrons consist determinant of different antibiotic resistance genes such as ß-lactams, aminoglycosides, chloramphenicol, rifampin, trimethoprim, and sulfonamides. Also, they play a considerable role in the mechanism of MDR genes transmission among Gram-negatives bacteria.[12] The aims of this study were to investigate the antibiotics susceptibility patterns, the frequency of MDR and the association of integrons with MDR and non-MDR among phylogenic groups UPEC strains were isolated from Azerbaijan, Iran.

Materials and Methods

Patient and isolates

A total number of 1793 clinically suspected as UTIs were studied by the urine culture. One hundred seventy-six non-duplicated *E. coli* isolates were collected from Azerbaijan (Tabriz, Urmia and Khoy) during 2016. One calibrated loopful of midstream urine was cultured on sheep blood agar and Mac-Conkey agar plates. The bacterial isolates with significant CFUs/mL growth ($\geq 10^5$ CFUs/mL) were included and identified by the standard microbiological methods including morphology of colony, Gram staining, oxidase, indole, urease, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, acid produced from lactose, and phenylalanine deaminase tests, culture in Eosin methylene blue (EMB), Mac-Conkey agar, triple sugar iron medium (TSI), Simmons citrate agar, SIM medium (Sulfide hydrogen, Iodole, Motility), Methyl Red and Voges-Proskauer (MR-VP).[13] This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC. 1395.847).

The disk diffusion agar

All isolates were tested for their susceptibility to 23 antimicrobial agents. The disk diffusion method was performed on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2012. Antibiotic disks used in this study included ampicillin (10µg), trimethoprim (5µg), cefazolin (30µg), nalidixic acid (30µg), moxifloxacin (5µg), ofloxacin (5µg), ciprofloxacin (5µg), levofloxacin (5µg), cefuroxime (30µg), aztreonam (30µg), ceftriaxone (30µg), cefotaxime (30µg), ceftazidime (30µg), gatifloxacin (5µg), tobramycin (10µg), gentamicin (10µg), cefepime (30µg), nitrofurantoin (300µg), amikacin (30µg), ertapenem (10µg), fosfomycin (200µg), meropenem (10µg) and imipenem (10µg).[14] In this study, MDR was defined as acquired resistance to at least one drug in three or more antimicrobial categories.[15]

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Phylogenetic grouping

A loopful of *E. coli* colony was suspended in 400 mL of TE buffer (10 mM Tris-HCL, 1 mM EDTA, and pH 8.0) and incubated at 80°C for 20 min to kill the organism. The DNA was extracted by the CTAB method.[16] Phylogenetic grouping was performed using the PCR for all isolates according to the method described by Clermont et al.[17] The multiplex-PCR reactions were performed for amplification of the *chuA*, *yjaA* genes and the DNA fragment TSPE4C2. The primer sequences were *chuA*, forward (5′-GACGAACCAACGGTCAGGAT-3′) and *chuA* reverse (5′-TGCCGCCAGTACCAAAGACA-3′), *yjaA* forward (5′-TGAAGTGTCAGGAGACGCTG-3′) and *yjaA* reverse (5′-ATGGAGAATGCGTTCCTCAAC-3′), and *tspE4C2* forward (5′-GAGTAATGTCGGGGCATTCA-3′) and *tspE4C2* reverse (5′-CGCGCCAACAAAGTATTACG-3′), which produce 279bp, 211bp, and 152bp fragments, respectively. The PCR was carried out under initial denaturation for 5 min at 94°C; following 30 cycles of 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C; and a final extension step of 7 min at 72°C. Gel electrophoresis was run on 2% agarose in 0.66 TBE buffer, stained with ethidium bromide and interpreted according to Clermont *et al*. (2000).[17]

Detection of integron-associated int genes

All *E. coli* strains were tested for the presence of different classes of integron-associated integrases by using the PCR method. After amplification, the PCR products were separated by running on 1.5 % agarose electrophoretically in 0.66 TBE buffer, stained with DNA safe stain and viewed under UV light. The selected primers for amplification of all three classes of integrase genes were *int1* forward, 5′-CAGTGGACATAAGCCTGTTC-3′, and *int1* reverse, 5′-CCCGAGGCATAGACTGTA-3′, for the class I integron-associated integrase; *int2* forward, 5′-CACGGATATGCGACAAAAAGGT-3′, and *int2* reverse, 5′-GATGACAACGAGTGACGAAATG-3′, for the class II integron-associated integrase; and *int3* forward, 5′-GCCTCCGGCAGCGACTTTCAG3′, and *int3* reverse, 5-ACGGATCTGCCAAACCTGACT-3′, for the class III integron-associated integrase, which amplified fragments of 160bp, 788bp and 979bp, respectively.[8] The PCR cycles were as follows: denaturation for 5 min at 94°C, 30 cycles of 45 s at 94°C and 45 s at 58°C, 45 s at 72°C and a final extension step of 5 min at 72°C.

Statistical analysis

Differences in the prevalence of integrase genes between phylogenetic groups, as well as between antimicrobial MDR isolates, were assessed by using χ^2 and Fisher's exact tests by the SPSS software (Washington, the USA), version 19. A P-value of ≤ 0.05 was considered statistically significant.

Results

In the present study, 176 *E. coli* isolated from the urine specimens of 118 (67%) females and 58 (33%) males, with a mean age of 52±21 years. The prevalence of UTIs due to *E. coli* was 70.1% (176 of 251). The patients were hospitalized in internal (65.9%), surgery (19.9%), Intensive Care Unit (ICU) (8.5%) and pediatric wards (5.7%). The high frequency of resistance was observed to cotrimoxazole (96.9%), ampicillin (85%), trimethoprim (80.1%) and cefazolin (79.6%). Carbapenems were the most effective antibiotics. All isolates were imipenem susceptible; however, one isolate (0.6%) and three isolates (1.7%) were meropenem and ertapenem resistant, respectively. Resistance to fosfomycin was observed in two isolates (1.2%). High and low-frequency resistance among cephalosporins was observed to cefazolin (79.6%) and cefepime (33%). Amikacin was the most effective agent among aminoglycosides (92.6%). According to the disk diffusion method, 140 isolates (79.5%) were MDR.

The majority of bacterial isolates (60.8%) belonged to the phylogenic group B2, followed by the D (26.1%), B1 (9.1%) and A (4%) groups. MDR isolates were found to closely associated with phylogenic group D (82%) (Table 1; Figures 1 and 2).

Integron presence was detected in 135 (76.7%) isolates. One hundred and twelve (63.6%) isolates were positive for only the class I integron-associated integrase, while 8 (4.5%) isolates were positive for only the class II integron-associated integrase. Both class I and II integron-associated integrase simultaneously were detected in 15 (8.5%) isolates. The class III integron-associated integrase was not found. MDR isolates were found to be significantly associated with

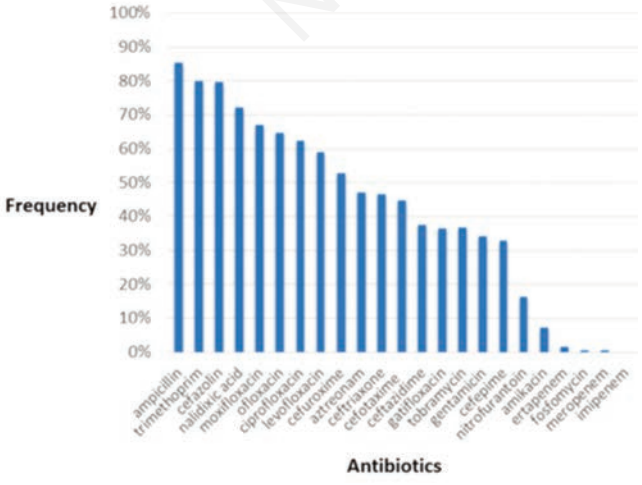

Figure 1. Antibiotics resistance patterns of bacterial isolates in this study.

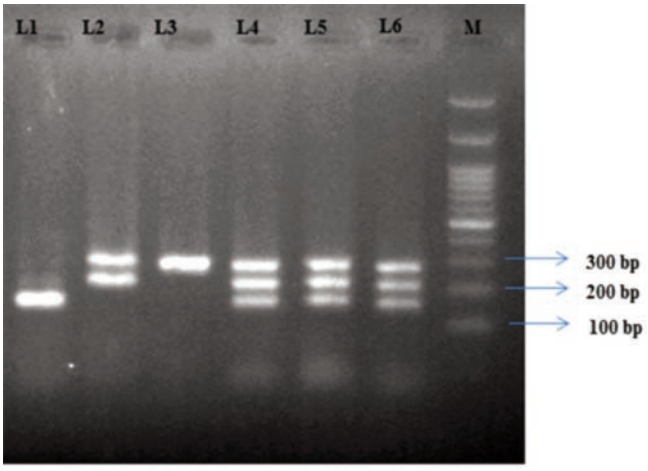

Figure 2. Multiplex PCR patterns for E. coli phylogenetic groups: Lane 2, 4, 5 and 6: group B2, Lane 3: group D and Lane 1: group B1. Lane M contained DNA size marker.

integrons I. The high frequency of integrons I and II were detected among the phylogenic group B1 (Table 2).

Discussion and Conclusions

E. coli is the most common pathogen isolated from a community or nosocomial UTIs.[3,4] Antibiotic resistant UPEC is increased especially in the healthcare settings, which makes it a major public health challenge. Therefore, it is very critical to determine the antibiotic susceptibility patterns of *E. coli* isolates for successful treatment and management.[18]

The emergence and spread of extended-spectrum β-lactamases (ESBLs) have limited the empirical administration of cephalosporins and penicillins. In the present study, a different level of resistance was detected against aminoglycoside, quinolones, cotrimoxazole and trimethoprim, cephalosporin and penicillins. According to the disk diffusion results, carbapenems and fosfomycin are the most effective agents against bacterial isolates. All isolates were imipenem sensitive. However, resistance rate to meropenem, ertapenem and fosfomycin are 1.7%, 0.6% and 1.2%, respectively. In most cases, the frequency of carbapenems and fosfomycin resistant *E. coli* isolated from UTIs were reported lower than 5%.[19-21] This may be due to the low use of carbapenems and fosfomycin in these countries.

The increasing prevalence of MDR *E. coli* strains complicate the management of infections and known as a serious challenge to public health.[22] According to the disk diffusion method, 140 isolates (79.5%) were MDR. Gonçalves *et al*. (2016)[23] from Brazil, Ibrahim *et al*. (2012)[24] from Sudan and Khawcharoenporn *et al*. (2013),[7] from Thailand reported 100%, 92.2% and 20.1% MDR *E. coli* from UTIs, respectively. MDR frequency may vary according to the geographical regions, previous exposure to antibiotics, the patient's characteristics, the social factors.[24] Consequently, for the successful treatment of UTIs, knowledge on antibiotic resistance patterns and resistance mechanisms is necessary.

The majority of bacterial isolates (60.8%) belonged to the phylogenic group B2. Similar to our study, other studies also were reported phylogroup B2 as the most frequent pathogens involved in *E. coli* isolated from UTIs.[25,26] These results are comparable to a study carried out by Cao from China. In the present study, the phylogenetic group D was the most frequent MDR *E. coli* isolates associated with UTIs that indicate that group D isolates may be prone to be more resistant to antimicrobials than other groups.[22] This is similar to a previous report which proved that resistance elements, such as β-lactamase, preferentially are associated with phylogroup D.[27]

We detected the presence of integrase in 135 (76.7%) isolates. Class I integron-associated integrase was detected in 63.6% isolates as the most common class of integron. Although 8 (4.5%) isolates were positive for the only class II integron-associated integrase, statistical analysis was shown a significant association between the presence of class I integron (alone or in the coexistence with class 2) and MDR (P-value ≤ 0.05).

Other researchers reported class I of integron as a frequent class and association of class I integron presence and MDR.[8,28] Class I of integron was most prevalent among phylogroup B1 than other groups (Table 1). Similar to another study, class III integrase was not detected among UPEC isolates.[8] There is no significant correlation between class II and MDR (P-value 0.486). Class II integrons are usually detectable in 4-20 % of UPEC isolates.[8,29] The class II integrase is nonfunctional, due to the presence of a premature in-frame stop codon. Barlow & Gobius (2006)[30] described an *E. coli* strain in which the stop codon had been substituted by a glutamine codon and recommended that the related IntII protein is efficient. They also reported that such functional integrons may characterize an additional mechanism by which resistance elements could be transferred in isolates.[30]

Our results indicate a high frequency of MDR UPEC in our health centers, which need appropriate programs to control the spread of these isolates. In the current study, the most effective antibiotics are carbapenems and fosfomycin. Therefore; these agents should be the last resort for the treatment of UTIs caused by MDR UPEC. Our findings show a significant correlation between MDR and the presence of class I integron in UPEC. We conclude that horizontal transmission of class 1 integron-association determinants plays an important role in the development of MDR UPEC, especially among phylogroup B1.

Table 1. Distribution of integrons and multidrug-resistant isolates (MDR) among phylogenetic groups.

Phylogenic groups (Number)	*int1* N (%)	*int2* N (%)	*int1+int2* N (%)	MDR N (%)
A (7)	4 (57.1)	0	0	5 (71.4)
B1 (16)	11 (68.8)	0	2 (12.5)	12 (75.0)
B2 (107)	69 (64.5)	6 (5.6)	8 (7.5)	85 (79.4)
D (46)	28 (60.9)	2 (4.3)	5 (10.9)	38 (82.6)
Total (176)	112 (63.6)	8 (4.5)	15 (8.5)	140 (79.5)

Table 2. The association between integrons and multidrug-resistant isolates.

Isolates	*int1 (%)*	*int2 (%)*	*int1+ int2 (%)*
MDR	87.5%	94.6%	100%
non-MDR	12.5%	5.4%	0
p-value	0.00	0.486	0.027

MDR, multidrug-resistant.

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