Original Article

Frequency of Class 1 Integrons among Escherichia coli Isolates of Patients with Urinary Tract Infection

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Abstract

Background: Recent studies demonstrated an increased pattern of drug resistance in uropathogenic Escherechia coli (E. coli) which is considered as the most common cause of urinary tract infections (UTIs). Present investigation was undertaken to evaluate antibiotic resistance pattern of E. coli causing UTIs obtained from urine samples and their relationship with integron class1. Apart from that, special emphasis was given on mediated and transferable antibiotic resistance in E. coli as well as the mobilized integrons that contribute to dissemination of antibiotic resistance.

Methods and Materials: Susceptibility of isolates to 12 antibiotics was tested by the Kirby -Bauser disk diffusion method. The sensitivity was monitored by zone of inhibition according to the clinical and laboratory standard institute (CLSI) guidelines. Plasmid DNA from E. coli strains was tested for class 1 integron by PCR.

Results: Rate of resistance to the 12 antibiotics is as follows: Ampicillin (89.4%), Cefotaxim (31%), Ciprofloxacin (22.4%), Aztreonam (21.7%), Ceftazidim (21.1%), Ceftriaxon (20.5%), Co-trimoxazole (19.9%), Gentamicin (15.5%), Amikacin (7.5%), Cefepim (11.8%), Nitrofurantoin (6.2) and Imipenem (1.9%). Existence of integron was confirmed in 41.9% of isolates. Significant association was evaluated by PCR between resistance to Gentamicin, Amikacin, Gentamicin, Amikacin, Cefotaxim, Ceftazidim, Ceftriaxon, Aztreonam, Ciprofloxacin and Co-trimoxazole with the existence of class 1 integrons.

Conclusion: Imipenem could be used as the initial therapy for E. coli in UTIs. Similar studies are essential to determine appropriate guidelines for empirical therapy which vary by location.

Key words: Urinary tract infections, E. coli, Class 1 integron, Drug resistance.

Introduction

Urinary tract infection (UTI) is the second common infection in human which affects millions of people each year (1). Increasing rates of resistance among bacterial uropathogens has caused growing concern in both developed and developing countries. Escherichia coli, the most common member of the family Enterobacteriaceae, accounts for 75-90% of all UTIs in both in- and out-patient settings (2).

Understanding molecular mechanisms, through which resistance genes transfer, may contribute to developing new antimicrobial agents as well as preventive measures to stop further dissemination of resistance determinants among pathogens (3).

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Many resistance genes in bacterial genomes and in extra-chromosomal pieces of DNA encode different mechanisms of drug resistance (4). Gene mutations can spread from cell to cell by mobile genetic elements such as transposons and integrons (5). Drug resistance genes can easily be transferred among isolates. Plasmids may affect bacterial virulence and antibiotic resistance and serve as epidemiological markers (5).

Class 1 integrons are genetic elements located on bacterial chromosome or plasmid while transposons often carry genetic determinants for antimicrobial drug resistance (6).

Dissemination of antibiotic resistance genes by horizontal transfer has led to rapid emergence of antibiotic resistance among clinical isolates of bacteria (7). Class 1 integrons are widely distributed among both nosocomial and community gram-negative isolates. The need for systematic epidemiologic studies on the role of class 1 integron in antimicrobial drug resistance in bacteria has recently been emphasized. The prevalence of class 1 integrons is high among gram-negative isolates in European patients (8, 9) and some bacteria carry multiple integrons.

To date, most of the resistance integrons found in clinical isolates of Enterobacteriaceae are of class 1 integrons, which are highly associated with resistance to antimicrobial agents (7). These reports suggest that class 1 integrons are relatively common, especially among Enterobacteriaceae, and they contribute to dissemination of antimicrobial drug resistance in healthcare settings. Integron structure contains essential elements for insertion and mobilization of gene cassette (7).

In present study, we analyzed the association between class1 integron and antibiotic resistance pattern in Escherichia coli isolates of urine samples obtained from patients referred to our institution.

Methods and materials:

Total number of 161 Escherichia coli samples were obtained from urine specimens of patients at Hajar Hospital (affiliated to Shahrekord University of Medical Sciences, Shahrekord, Iran) between April and August 2010 by standard bacteriological methods. The drug susceptibility tests were done for all isolates by Kirby-Bauser agar disk diffusion method as recommended by CLSI (10) on Muler-Hinton agar medium (Merck, Germany) for antimicrobial disks (Mast Co.) including Gentamicin (10µg), Amikacin (30µg) ,Ceftriaxon (10 µg), Aztreonam (30 µg), Cefepim (20 µg), Ceftazidim (30µg), Ampicillin)20 µg), Imipenem (10 µg) , Ciprofloxacin (5 µg), Co-trimoxazole (25 µg) and Nitrofurantoin (300 µg) (10). DNA was extracted by using of Sinagene DNPTM kit (Sinagene Co, Tehran, Iran). Integron Class 1 was detected using PCR targeting conserved sequences of class 1 integron (intI1), as adapted from Ebner et al (2004). Primer pairs were purchased from a commercial source (DNPTM Sinagene). Twenty-five µl of reaction mastermix was used composed of 2.5 Mm of 10x PCR, 1.5Mm of 50 Mm MgCl2, 2.5 Mm dNTP, 0.5 Mm of primer (forward and reverse) together with 0.1 unit of Taq DNA polymerase (5 U/ml) primers (reported from 5′ to 3′) comprised Inti1-F';TCTCGGGTAACATCAAAG-3'andIntI-R:5'-AGGAGATCCGAAGACCTC-3' (11). Amplification specification were as follows: 4 min at 94º C followed by 30 cycles of 1 min at 94º C, 45 min at 55º C ,30s at 72º C and 5 min at 72º C. PCR products were analyzed by acrylamid gel electrophoresis. The expected size (241bp) was ascertained by electrophoresis in polyacrylamid gel with appropriate molecular size markers (100bp DNA ladder). E. coli (ATCC25922) was used as a control for determination of antibiotic susceptibility and PCR amplification. The analysis of the findings was performed by Chi-Square test and Statistical Package for Social Sciences (SPSS). P-value of ≤0.05 was considered as statistically significant.

Results

coli isolated from 161 urine samples of patients of which 72.7% (n = 117) were female and 27.3% (n = 44) were male. Rate of resistance to 12 antibiotics was as followed; Ampicillin (89.4%), Cefotaxim (31%), Ciprofloxacin (22.4%), Aztreonam (21.7%), Ceftazidim (21.1%), Ceftriaxon (20.5%), Co-trimoxazole (19.9%), Gentamicin (15.5%) Amikacin (7.5%), Cefepim (11.8%), Nitrofurantoin (6.2) and Imipenem (1.9%) (figure 1). The highest and lowest levels of resistance were to Ampicillin and Imipenem, respectively. Frequencies of resistance to three, four, five and six or more antibiotics were 37 (35.5%), 22 (21.1%), 19 (18.2%) and 26 (25%), respectively. The existence of class 1 integron was confirmed for 79 (49.1%) of isolates by PCR (figure 2). Association of drug resistance to Amikacin, Gentamicin, Cefotaxim, Ceftazidim, Ceftriaxon, Aztreonam, Ciprofloxacin and cotrimoxazole with existence of class1 integron was statically significant (table 1).

Discussion

Objective of the present study was to investigate the drug resistance pattern and the frequency of class 1 integron as well as the relationship between antibiotic resistance and class 1 integron of the E. coli isolates in patients with UTI. Isolates in this study were highly sensitive to Imipenem. Extreme sensitivity of E. coli isolates to Imipenem has been earlier reported by Tariq et al (18(. Furthermore, this antibiotic has been recently administrated in clinical practice.

Quinolones (ciprofloxacin for instance) are often prescribed to treat gastrointestinal infections and UTI. Therefore, it could be expected that the prevalence of strains resistant to these antibiotics is increasing gradually. A consistent stepwise increase in E. coli resistance to Ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by Bolon et al (19). Ciprofloxacin resistance was 25.8% in Portugal and 24.3% in Italy while in Germany and the Netherlands it was 15.2% and 6.8%, respectively (20). Ciprofloxacin resistance in our isolates was 21%, which was similar to that in Southern European countries.

Previous studies showed associations between drug resistance genes and class 1 integron in bacteria responsible for nosocomial outbreaks (21). Class 1 integrons are frequently reported in clinical isolates of Enterobacteriaceae. As reported in other recent studies, resistance to Quinolones was more common among integrons-containing strains (22).

To alleviate this suffering situation in developing nations, clinicians should prescribe antibiotics wisely and sufficiently and there should be periodic supervisions on drug consumption by state organizations (23). It is worth mentioning that in present study, 64.6% of samples were resistant to three or more antibiotics, most noticeably to Ampicillin. Different resistance rates are from Iran. It was 65% in recent years (23, 25) and 10.9% in Kashan (26). Varying resistance rates in different countries indicates how prescriptions are controlled. We found the minimum resistance for Imipenem and Amikacin. Tariq, Mathai and Adwan, also reported the high sensitivity of Imipenem as the drug of choice for UTI)18, 24, 25(. The results of this study showed a significant association between overuse of antibiotics and the emergence of resistance in E. coli in UTI. Widespread use of these agents has contributed to the rise of bacterial Quinolone resistance. Fluoroquinolones should thus be used prudently while avoiding unnecessary prescriptions and considering alternative regimens for UTI. The widespread dissemination of resistant organisms would severely limit therapeutic options for physicians facing these organisms, because Carbapenems are the only drugs uniformly active against these organisms. The increased risk of resistance of E. coli in hospitalized patients should be considered as an alarm for choosing alternative therapies for community-acquired UTIs. It is important that these new antimicrobial agents should be used sparingly and with discretion. Furthermore, continuous monitoring of the antibiotic susceptibility of

Carbapenems is also necessary to check the effectiveness of this medication. The results of such study would be beneficial for determining guidelines for empirical therapy regimens. This is an important consideration reflected the fact that inappropriate choice of empirical antibiotics has been associated with poor outcomes and higher mortality rates in patients infected with E. coli.

Class 1 integrons play an important role in antibiotic resistance of clinical E. coli strains because they are able to capture, integrate and express gene cassettes encoding certain antibiotic resistance (16). The presence of integrons in E. coli isolates is a serious risk factor for spreading antimicrobial resistance. The prevalence of integrons ranging from 22% to 59% which has been reported from clinical E. coli isolates in several previous studies on UPEC in Europe and Asia (6, 15). We also observed a similar trend of prevalence of class 1 integron (49.01%) in our study. In our isolates, class 1 integrons were significantly associated with resistance to certain antibiotics including Gentamicin, Ceftriaxon, Aztreonam, Ciprofloxacin and Co-trimoxazole which is comparable with similar studies in south and northern west of Iran (27, 28).

Our findings also suggested that urine cultures and susceptibility tests cannot be neglected to avoid worrisome trend of developing resistance to most commonly administered antimicrobials used for treatment of UTIs.

Conclusion

Most of our E. coli isolates showed multiple antibiotic resistance. Maximum resistance was found against Ampicillin whereas minimum resistance was detected against Imipenem. Hence Imipenem might be the drug of choice to treat UTI. The results presented in this study could also help the establishment and enforcement of infection control measures.

Figure 1. Antibiotic resistance/sensitivity pattern of Escherichia coli isolates (n = 161) recovered from UTI patients

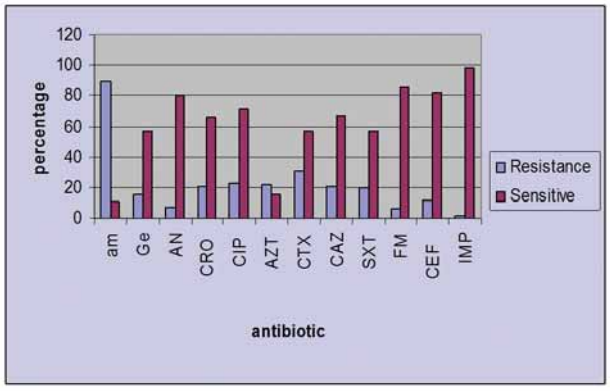
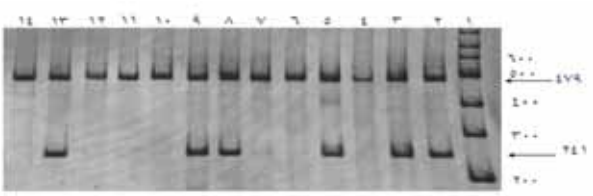


Figure 2: Class 1 integron (int1; 241bp) PCR of Escherichia coli Lane1:marker lane(2,3,5,8,9) intI +) lane13,14 positive, negative control –lane 1-1316sRrNA(479bp)



Antibiotic	integron	Positive		Negative		Total		Significant
		NO	%	NO	%	NO	%	
Ceftazidim	resistant	24	70.6	10	29.4	34	100	0.003
	sensitive	43	39.8	65	60.2	108	100	
Cefotaxim	resistant	22	71	9	29	31	100	.000
	sensitive	32	35.2	59	64.8	91	100	
Ceftriaxon	resistant	26	78.8	7	21.2	33	100	0.000
	sensitive	40	37.7	66	62.3	106	100	
Aztreonam	resistant	22	62.9	13	37.1	35	100	0.000
	sensitive	43	39.8	65	60.2	25	100	
Cefepim	resistant	10	52.6	9	47.4	19	100	0.5
	sensitive	63	47.4	70	62.6	133	100	
Ciprofloxacin	resistant	43	37.4	47	11.4	38	100	0.000
	sensitive	49	8.39	74	2.60	90	100	
Ampicillin	resistant	73	50.7	71	49.3	144	100	0.17
	sensitive	2	6.28	5	4.71	7	100	
Amikacin	resistant	9	75	3	25	12	100	0.48
	sensitive	62	48.1	67	51.9	196	100	
Gentamicin	resistant	19	76	76	55.9	25	100	0.003
	sensitive	60	44.1	76	3.64	136	100	
Nitrofurantoin	resistant	79	70	3	30	100	100	0.22
	sensitive	64	44.6	74	53.6	138	100	
co-trimoxazole	resistant	25	78.1	7	21.9	32	100	000
	sensitive	29	31.9	62	68.1	91	100	
Imipenem	resistant	2	66.7	1	33.3	3	100	0.48
	Sensitive	77	48.4	81	51.3	158	100	

Table1: Association between integrons and antibiotic resistance in 161 Escherichia coli isolates.

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