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Frequency of Class 1 Integron in *Escherichia Coli* Strains Isolated from Patients with Urinary Tract Infections in North of Iran

Fatemeh Ahangarkani[1], Ramazan Rajabnia[2] , Elaheh Ferdosi Shahandashti[3],Meghdad Bagheri[3] , Maryam Ramez[3]

[1]Antimicrobial Resistance Research Center, Department of Infectious Diseases, Mazandaran University of Medical Sciences, Sari, Iran
[2]Infectious Diseases Research Center, Babol University of Medical Sciences, Babol, Iran
[3]Infectious Diseases Research Center. Babol University of Medical Sciences, Babol, Ira[n]

Corresponding author: Maryam Ramez. Infectious Diseases Research Center, Babol University of Medical Sciences. Ganjafroz street, Babol, Iran. Phone: +981132199936. E-mail: antimicrobialresistence0111@gmail.com

ABSTRACT

Background: Due to the importance of antibiotic resistance in *E.coli* and the possible role of integrons in creating of resistance, this study was performed to survey of class 1 integron in *E. coli* strains and their resistance to three routinely used antibiotics. **Methods**: In this cross-sectional study, 100 strains of *E. coli* were isolated from patients with Urinary tract infection. After diagnosis of bacteria, genomes were extracted. Then, presence of integron class 1 was evaluated by using PCR. Antibiotic susceptibility testing method, the micro dilution broth was performed according to the standard CLSI2010. Data were analyzed using SPSS16 software. **Result**: Out of the total number of 100 *E. coli* cases, 22 cases (22%) had class 1 integron. Resistance against cotrimoxazol, cefixime and ciprofloxacin antibiotics were 67%, 34% and 34% respectively. In 22 *E. coli* cases positive for integron class1 gene, resistance against three antibiotics were 100%, 95.45% and 90.90% respectively, which is statistically significant ($p < 0.05$). **Conclusion**: Resistance level against antibiotics in samples containing class 1 integron were significantly higher than those lacking this gene, which may be confirm the present of class 1 integron in creation of clinical strains with resistance to this antibiotics. Using suitable antibiotics may be preventing transmission of resistance genes through integrons.

Key words: *E. coli,* **class 1 integron, Urinary Tract Infection, antibiotic resistance.**

1. BACKGROUND

Urinary Tract Infection (UTI) is one of the most common infections, and account for significant morbidity and high medical costs (1). Many bacteria can cause infection in urinary tract and E. coli is the most common agent (2). Basis of a suitable treatment in urinary infection is choosing of an antibiotic with high efficiency and low cost; and the main problem in the treatment of UTIs caused by E. coli is resistance of this bacterium against a great number of common antibiotics. On the other hand, the spread of antibiotic resistance is almost always associated with increased use of antibiotics (3).

Integrons are one of the mobile genetic elements which are able to carry genes of resistance to different antibiotics (4). These elements are found in different locations of plasmids and chromosome. Integrons are able to surrounding the genes, and displace them while they are located within the gene cassettes (5, 6). Integrons contain integrase gene, two conserve areas of *sul1* and *int1*, and one variable area of gene cassettes (7). Integrons are divided into four classes based on the type of integrase genes, between which, class 1is the more studied and prevalent (6). Since integrons can be located on the plasmids and transposons, they can spread between bacterial species rapidly. Resistance genes, which are located in the gene cassettes, can be separated and be entered to other integrons. This is an important phenomenon in the creation and distribution of new resistance cassettes and development of plasmids and transposons. Importance of association of multidrug resistance and presence of integron, play an important role in development of multiple resistance (8). The percentages of integron distributions in uropathogenic E. coli are variable (9).

Regarding to clinical importance of class 1 integron in antibiotic resistance and there is no reports available on prevalence of integrons class 1 in *E. coli* in north of Iran. This study describes to molecular detection of class 1 integron in *E. coli isolated* from UTI, and its resistance to cotrimoxazol, cefixime and ciprofloxacin antibiotics by microdilution method.

2. MATERIAL AND METHODS

This cross-sectional study was performed on 100 cases of isolated *E. coli*, from UTI. Urine samples were taken from hospitalized patients in north of Iran. Samples were collected properly and cultured on sheep blood agar (Merck- Germany) and eosin

methylene blue agar (Merck- Germany) immediately. Then the media were incubated at 37 °C for 18- 24 hours. Clinical isolates of E. coli were identified by standard methods such as colonial/ microscopic morphology and enzymatic characteristics (10).

2.1. Antibiotic susceptibility test

Susceptibility of the clinical isolates to three routinely used antibiotics was determined by standard broth dilution (microdilution) technique. MIC determined according to the recommendations of the standard protocol of CLSI 2010 (11). The antibiotics used, were cotrimoxazol, cefixime and ciprofloxacin.

2.2. DNA extraction and PCR amplification

To extract DNA, High pure PCR template preparation kit was used from Roche Company (Germany). To amplification int1 region, the set of primers, which sequences were int1 F: 5' TCTCGGGTAACATCAAGG 3' and int1R: 5' AGGAGATCCGAAGACCTC 3' were used (8) (Manufactured by Copenhagen Company- Denmark). PCR reaction was performed in a final volume of 50 µl including 30 µl de-ionized water, 50 mMKCl, and 50 mMTrisHCl, 0.2 pmol/L from each primer, 2.0 mMdNTPs, 1.5 U of Taq DNA polymerase enzymes, 2 mM MgCl2 and 400 ng of DNA sample. Amplification reactions for int1 gene, includes of primary denaturation at 94 °C for 5 min, and then followed by 35 cycles of denaturation at 94 °C for 1, annealing at 55 °C for 1 min and extension at 72 °C for 30 seconds. One cycle for the final extension at 72 °C for 5 min was done too. PCR reaction was conducted at the presence of positive and negative controls.

2.3. Gel Electrophoresis

After performance PCR reaction, electrophoresis of PCR products was carried out in 1.5% agarose gel for 45 min and voltage 70. Then, results were evaluated under UV light on the UV Trans illuminator.

2.4. Statistical analysis used

Data were analyzed using SPSS16 software and CHI-square test, and the P value <0.05 was considered as significant.

3. RESULTS

Total of 128 bacteria isolated from UTI, 100 isolates (78%) were E. coli, which is showing the prevalence of these bacteria in UTI. Using susceptibility determination test by the microdilution method, the percentage of isolates resistant to the antibiotics cotrimoxazol, cefixime and ciprofloxacin, were 67%, 34% and 34%; and the percentage of isolates sensitive to these antibiotics were 22%, 58% and 51% respectively (Table 1).

Antibiotic	MIC result	Percentage	Integron class 1 gene positive (%)
Cefexime	Resistant	34	61.76
	Intermediate	15	6.66
	Sensitive	51	0
Ciprofloxacin	Resistant	34	58.82
	Intermediate	8	25
	Sensitive	58	0
Cotrimoxazol	Resistant	67	32.83
	Intermediate	11	0
	Sensitive	22	0

Table 1. Relation between susceptibility to various antibiotics and presence of integron class 1 gene in E.coli.

During the PCR reaction, the 241 base pair band was observed, that shows the int1 gene amplification in PCR reaction

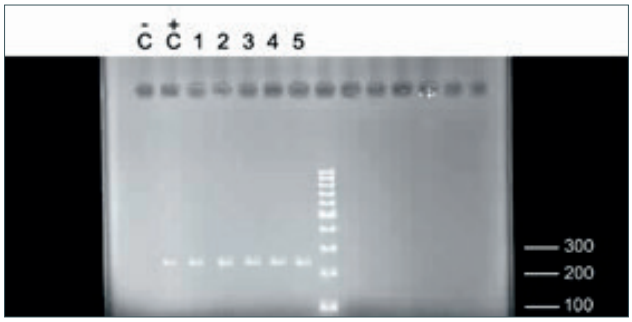

Figure 1. The result of electrophoresis of the PCR product ; C- as negative control , C+ as positive control and numbers 1 , 2 , 3 , 4 and 5 as samples containing class 1 integron genes

was done (fig.1). Out of the 100 E. coli strains, 22 isolates (22%) were contained the class 1 integron gene.

Out of the 34 isolates resistant to cefixime and ciprofloxacin, 21 (61.76%) and 20 isolates (58.82%) were contained class 1 integron gene respectively. It is noteworthy that all of the isolates containing class 1 integron were not sensitive to cefixime and ciprofloxacin. Out of the 67 isolates resistant to cotrimoxazol, 22 isolates (32.83%) contained class 1 integron gene. In other words all of the isolates containing class 1 integron were resistant to cotrimoxazol (table 1).

In 22 *E. coli* cases positive for integron, resistance against cotrimoxazol, cefixime and ciprofloxacin antibiotics were 100%, 95.45% and 90.90% respectively. The relationship between the presences of class 1 integron and resistance to three antibiotics is statistically significant ($p < 0.05$).

In Table 1, MIC ≥ 4 µgr/ml was considered resistant and MIC ≤ 1 µgr/ml was sensitive for antibiotics cefixime and ciprofloxacin; and MIC >8.152 µgr/ml was considered resistant and MIC < 2.23 µgr/ml was sensitive for antibiotic cotrimoxazol.

4. DISCUSSION

Our study showed that (22%) *E. coli* studied, were contained class 1 integron gene. 95% of the isolates containing this gene were resistant to cotrimoxazol, cefixime and ciprofloxacin antibiotics; and there was significantly relationship between integron and resistance to them ($p < 0.05$). Prevalence of class 1 integron in *E. coli* in different studies was 22 to 59 percent (7,12-14). In a study by Rao et al showed that 49 % of *E.coli* was contained class 1 integron gene (8). In another study in Taiwan, Chang et al show, totally, 54 isolates (52%) contained class 1 integron (15).

Prevalence of integrons in these studies was higher than our study, which can be due to the differences in the prevalence of class 1 integron gene in different geographic areas, the number of samples and uncontrolled use of antibiotics.

In another study in south of Iran 16.6% of uropathogenic *Escherichia coli* strains were contained class 1 integron gene (9). Considering the low prevalence of integrons in our study, may suppose that the resistance genes cassettes could be carried on the other transposable elements such as transposons or prophages rather than integrons, although in the strains studied integrons were significantly associated with resistance to certain antibiotics co-trimoxazol, cefixime and ciprofloxacin.

In Singh R et al study 43 isolates (16%) contained class 1 integron gene. The isolated bacteria containing integron also were resistant to ciprofloxacin and cotrimoxazol, as the same to

our study (16). The percentage of ciprofloxacin (34%) and cotrimoxazol (67%) resistance observed in this study was which is on the high side in comparison with other studied (9, 17,18). Ciprofloxacin resistance in other studies was different (19). Chronic conditions and healthcare-associated factors are related to resistance to both fluoroquinolones and cephalosporins in patients with UTI (20). Increasing of antibiotics resistance in this study may be due to an irrational consumption rate of antibiotics and food from animal that have received antibiotics, transmission of resistant isolates between people ,self medication and non – compliance with medication. In this study were not certain limitations.

5. CONCLUSION

In this study, all bacteria containing class 1 integron gene were resistant or intermediate to routinely used antibiotics for treatment of UTI caused by *E.coli* . Resistance to these antibiotics is dangerous and warns that the antibiotic usage policy for infections due to this bacterium should be changed. Using suitable antibiotics may be preventing transmission of resistance genes through integrons.

CONFLICT OF INTEREST: NONE DECLARED.

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