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

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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 class 1 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 1 is 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).

1. 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 eosi

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were 22%, 58% and 51% respectively (Table 1).

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 used, were cotrimoxazol, cefixime and ciprofloxacin. Out of the 67 isolates resistant to cotrimoxazol, 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).

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 prevalence 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 phages 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

During the PCR reaction, the 241 base pair band was observed, that shows the int1 gene amplification in PCR reaction was done (fig.1). Out of the 100 E. coli strains, 22 isolates (22%) were contained the class 1 integron gene.

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

| Antibiotic          | MIC result | Percentage | Integron class 1 gene positive (%) |
|---------------------|------------|------------|-----------------------------------|
| Cefixime            | 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                                 |

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 gene.
our study (16). The percentage of ciprofloxacin (34%) and co-
trimoxazol (67%) resistance observed in this study was which
is on the high side in comparison with other studied (9, 17,18).
Chronic conditions and healthcare-associated factors are re-
lated 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 medica-
tion 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 antibi-
otics 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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