Class I integrons among multidrug resistant Enterobacter spp. isolates from hospitalized patients in Babol, North of Iran

Abstract

Background: Multidrug resistance (MDR) in Enterobacter spp. has created therapeutic challenges all over the world. The present study was conducted for evaluating the prevalence of class I integron, determining the gene cassettes and antimicrobial resistance profile of Enterobacter spp. isolates from clinical samples in Babol, North of Iran.

Methods: During a 13-month period, 30 Enterobacter spp. isolates were collected from Ayatollah Rouhani Hospital, Babol, Iran. Various types of antimicrobial agents were used to determine the resistance pattern. Class I integron and associated gene cassettes were detected by PCR assay.

Results: The resistance rates to AP, CPM, CTX, TM, NI, IMI, AK, CIP and GM antimicrobials were 100%, 93.3%, 33.3%, 33.3%, 30%, 20%, 20%, 20% and 13.3%, respectively. The distribution results of int genes showed that 63.3% of isolates carried the intI genes. Also, the prevalence of aadB, dfrA1, blaOXA30 and blaQSE1 genes were estimated at 36.66%, 33.3%, 6.6% and 0%, respectively.

Conclusion: Our results showed that class I integrons have a widespread distribution among the Enterobacter spp. isolates and have clinical relevance to MDR isolates. The results confirmed the necessity for uninterrupted monitoring to prevent distribution of multidrug resistance among Enterobacter spp. strains in Iran.

Keywords: Class I integron, Enterobacter, Multidrug resistance (MDR).

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Enterobacter spp. are aerobic gram-negative bacilli from Enterobacteriaceae family (1). These organisms have three major species (Enterobacter Cloacae, Enterobacter sakazii and Enterobacter Aerogenes). Enterobacter spp. are present in the environment and they are one of the most important opportunistic pathogen responsible for various hospitalized and community-acquired infections (2, 3), including pneumonia, bacteremia, septicemia, meningitis as well as the wound, urinary tract, respiratory tract and lower gastrointestinal infections (4, 5). The widespread and inappropriate use of antimicrobials applied for the treatment of bacterial infections, lead to a perilous increasing in resistant isolates and represents a great concern to the medical community (6-8). The acquisition of antimicrobial resistance in Enterobacter spp. may be related to mobile genetic elements (MGEs) such as plasmids, transposons and gene cassettes in integrons (8, 9). Integrons are conserved sequences carrying the antimicrobial resistance determinants while they are located within the gene cassettes (10-12). Integrons are constituted from three parts based on their conserved sequences (3’-CS and 5’-CS).
These genetic fragments are considered by the presence of a recombination site (attI), a promoter (Pc), and an intI gene (integrase) (13). Integrons are classified as four main classes through the type of integrase genes. Transferable class I integrons are the most prevalent among other classes (14). Class I integrons consist some resistance genes including AMEs (disinfectants and aminoglycoside-modifying enzymes), sulI gene (sulfonamide), dfr gene (dihydrofolavonol-4-reductase/trimethoprim), broad-spectrum b-lactamase and qacED1 (tetravalent ammonium compounds) (15) and carry various gene cassettes, e.g. aadB, dfrA1, blaOXA30 and blapSE1. Also, integrons are associated with MDR and play a critical role in the development of multidrug resistance (11). There are some reports dealing on the role of class I integrons in antibiotic resistance of Enterobacter spp. The results of Mokracka et al. established the role of Class I integron gene cassettes in resistance patterns of patient-isolated Enterobacter cloacae (16).

The knowledge about antibiotic resistance and its mechanisms is a way to reduce the risk of antibiotic therapy failure. The aim of this study was to detect the dissemination of class I integrons and gene cassettes in clinical isolates of Enterobacter spp. from Babol, North of Iran.

Methods

Sampling and identification: In this cross-sectional study, 30 clinical isolates of Enterobacter spp. were collected from clinical samples of patients admitted with urinary tract infections (UTI) at Ayatollah Rouhani Hospital, Babol, North of Iran. Enterobacter spp. were identified based on microbial bio-typing tests. All strains were stored in Luria-Bertani broth (Merck, Germany) containing 20% glycerol at -80°C for further use.

Antibiotic susceptibility test: According to the guidelines of Clinical and Laboratory Standards Institute (CLSI document M100-S25) (17), antimicrobial susceptibility tests were conducted on the Mueller-Hinton agar plates (Merck, Germany) through the standard disk agar diffusion method for antimicrobials, i.e. cefepime (CPM, 30 µg), gentamicin (GM, 10 µg), ciprofloxacin (CIP, 5 µg), imipenem (IMI 10 µg), amikacin (AK, 30 µg), cefotaxime (CTX, 30 µg), ampicillin (AP, µg), trimethoprim (TM, 5 µg) and nitrofurantoin (NI, 100 µg) (MAST Diagnostics, Merseyside, UK). Escherichia coli strain (ATCC 25922) was used as a positive quality control.

Polymerase chain reaction method: According to DNA purification kit (Roche, Germany) protocol, genomic DNA was extracted from expanded Enterobacter spp. colonies. The total volume of PCR reaction mixture was 25 µl, containing 0.7 µl of Taq DNA polymerase (5 IU/µl) (Amplicon Co., Denmark), 2.0 µl of PCR buffer, 1.0 µl of extracted DNA template, 0.5 µl of each primers (10 pmol), 0.6 µl MgCl2 (50 mM), 0.6 µl dNTPs (10 mM), and 19.6 µl ddH2O. Amplification was performed in a thermocycler (Corbett, Australia) as mentioned (table 1).

Statistical Analysis: All statistical analysis was made by SPSS Version 16. The correlation of class I integrons and antibiotic resistance was examined by chi-square test.

Table 1: Oligonucleotide primers used for PCR amplification.

| Gene | Sequence (5’-3’) | Cycles No. | PCR condition | Product size(bp) | Reference |
|------|-----------------|------------|---------------|------------------|-----------|
| attI | F-TCTCGGGTAACATCAAGG | 35 | Denaturation: 94 °C | 243 | (24) |
|      | R-AGGAGATCCGAAGACCTC |  | Time: 60 Sec | | |
| aadB | F-GGGCCGCTCATGGAGGATTT | 35 | Denaturation: 94 °C | 329 | (25) |
|      | R-TATCGGCACCTGAAGCGGC |  | Time: 60 Sec | | |
| dfrA1 | F-GGAGTGCCAAAGGTGAACAGC | 35 | Denaturation: 94 °C | 367 | (26) |
|      | R-GAGGCCAGATTGCTGGGTAACAC |  | Time: 60 Sec | | |
| blapSE1 | F-GGCAGCGATCATACACTCG | 35 | Denaturation: 94 °C | 153 | (8) |
|      | R-AATTCAATCCGCATACGTCGAT |  | Time: 30 Sec | | |
| blaoxa10 | F-ATTATCTACAGCGCCAGTGCATC | 35 | Denaturation: 94 °C | 716 | (27) |
|      | R-TTCGACCACAGTTTCTCAGAAGTC |  | Time: 30 Sec | | |


Results

**Bacterial Isolation:** In this study, 30 clinical isolates of *Enterobacter* were collected from the hospital-admitted patients from 12 (40%) males and 18 (60%) females.

**Antibiotic Resistance Profile:** In antibiogram screening, the resistance rates to AP, CPM, CTX, TM, NI, IMI, AK, CIP and GM antimicrobials were 100%, 93.3%, 33.3%, 33.3%, 30%, 20%, 20%, 20% and 13.3%, respectively (table 2). As our results, 93.33% of isolates contained MDR phenotype. The highest resistance rate was related to AP and CPE antimicrobials and the highest susceptibility was related to GM and AK antimicrobials (table 3).

**Class I integron and gene cassettes:** *IntI* gene was detected in 63.3% (n=19) of *Enterobacter* spp. isolates. The prevalence of *dfrA1, aadB, bla-OXA30* and *blaPSE1* genes were estimated at 11 (36.6%) 10 (33.3%), 2 (6.6%) and 0 (0%), respectively (table 2).

| Antibiotics | Total (n=30) | Integron Positive (n=19) | Integron Negative (n=11) | P<0.05 |
|-------------|--------------|--------------------------|--------------------------|--------|
|             | R No.(%) | I No.(%) | S No.(%) | R No.(%) | S No.(%) | R No.(%) | S No.(%) |        |
| Ampicillin  | 30(100%) | 0(0%)   | 0(0%)   | 19(100%) | 0(0%)   | 11(100%) | 0(0%)   | 0.181  |
| Cefepime    | 28(93.3%)| 2(6.6%) | 0(0%)   | 19(100%) | 0(0%)   | 10(90%)  | 1(9.1%) | 0.181  |
| Cefotaxime  | 10(33.3%)| 5(16.6%)| 15(50%) | 8(42.1%) | 11(57.9%)| 6(54.5%) | 5(45.5%)| 0.510  |
| Trimethoprim| 10(33.3%)| 1(3.3%) | 19(63.3%)| 6(31.6%) | 13(68.6%)| 5(45.5%) | 6(54.5%)| 0.447  |
| Nitrofurantoin| 9(30%) | 6(20%)  | 15(50%) | 9(47.4%) | 10(52.6%)| 6(54.5%) | 5(45.5%)| 0.705  |
| Imipenem    | 6(20%)   | 3(10%)  | 21(70%) | 5(26.3%) | 14(73.7%)| 4(36.4%) | 7(63.6%)| 0.563  |
| Amikacin    | 6(20%)   | 0(0%)   | 24(80%) | 4(21.1%) | 15(78.9%)| 2(18.2%) | 9(81.8%)| 0.850  |
| Ciprofloxacin| 6(20%)  | 9(30%)  | 15(50%) | 8(42.1%) | 11(57.9%)| 7(63.3%) | 4(36.4%)| 0.256  |
| Gentamicin  | 4(13.3%) | 1(3.3%) | 28(83.3%)| 4(21.1%) | 2(18.2%) | 15(78.9%)| 9(81.8%)| 0.850  |

| Type of resistant | Pattern of antibiotic resistant | No. of isolates | Total |
|-------------------|---------------------------------|----------------|-------|
| Resistant to 1 agent | AP                              | 2              | 2     |
| Resistant to 2 agents | AP-CPM                          | 11             | 12    |
|                    | AM-NI                           | 1              |       |
| Resistant to 3 agents | CIP-AP-NI                        | 2              | 6     |
|                    | CPM-AP-NI                        | 1              |       |
|                    | CPM-AP-AK                        | 2              |       |
|                    | TM-CIP-AP                        | 1              |       |
| Resistant to 4 agents | TM-CIP-CTX-AP                   | 2              | 2     |
| Resistant to 5 agents | TM-CIP-CTX-AP-NI                | 1              | 2     |
|                    | CTX-CIP-AP-IMI-AK                | 1              |       |
| Resistant to 8 agents | TM-CMP-CTX-CIP-GM-AP-IMI-AK     | 1              |       |
|                    | TM-CMP-CTX-CIP-GM-AP-IMI-NI     | 2              | 4     |
|                    | TM-CMP-CTX-CIP-GM-IMI-AK-NI     | 1              |       |
| Resistant to 9 agents | NI-IMI-AK-AP-GM-CIP-CTX-CPM-TM | 2              | 2     |
| Total              |                                  | 14             | 30    |

AP: ampicillin. CPM: cefepime. CTX: cefotaxime. TM: trimethoprim. NI: nitrofurantoin. IMI: imipenem. AK: amikacin. CIP: ciprofloxacin. GM: gentamicin.
Discussion

In recent years, MDR Enterobacter spp. has been increasingly reported as a cause of MDR nosocomial infections (18, 19). Increase in number of MDR strains has many reasons, i.e. inappropriate administering of antibiotics or transferring of resistance genes through MGEs (including transposons (TEs), integrons (int), plasmids and bacteriophages) (20).

In agreement with our results, several studies established that antibiotic resistance in Enterobacter spp. has increased due to misuse, overuse and uncontrolled use of antimicrobials, so 93.33% of tested Enterobacter spp. indicated MDR phenotype. Also, Rizk et al. (2), showed that 85.7% of strains were MDR.

All of the strains were resistant to AP antimicrobial and 93.3% of strains were resistant to CPM antimicrobial. Also, 42.8% of MDR E. coli isolates, 90% of MDR K. pneumoniae isolates and 25% of MDR Enterobacter spp. carried class I integron. They reported a significant correlation between the GM antimicrobial resistance pattern and class I integron in MDR E. coli isolates.

The highest susceptibility to GM antimicrobial was estimated 13.3%. These data are similar to Mansuri et al.’s (2, 21). Of 30 Enterobacter spp. isolates, 19 (63.3%) strains carried the intI gene. Rizk et al.’s (2), Amin et al.’s (1) and Zheng et al.’s studies (22) showed that 28%, 47.4% and 77.8% of strains contain intI gene, respectively. These differences are due to the geographical distribution, type of organisms and source of infections; such finding was observed in foreign countries as well (23). The prevalence of dfrA1, aadB, blaOXA30 and blaPSEI gene cassettes were 36.6% (11), 33.3% (10), 6.6% (2) and 0% (0), respectively. Also, no significant correlations were found between these gene cassettes and integrons with antibiotic resistance.

Our results showed that class I integron has a widespread distribution among Enterobacter spp. isolates and have clinical relevance to MDR isolates. The findings emphasized that there is an urgent requirement for continuous surveillance to prevent more distribution of emerging multidrug resistance among Enterobacter spp. strains in Iran.

Finally, it can be concluded that distribution of class I integron in Enterobacter spp. isolates is very high among hospitalized samples as a high impact factor in occurrence of multidrug resistance. Therefore, the knowledge of resistance pattern in strains is helpful for the correction of antimicrobial administration and prevention of further antibiotic resistance.

Author contributions

P.S, M.R and E.F.Sh conceived the study; M.R, A.F.Sh, and E.F.Sh collected all data; A. M, P.S and E.F.Sh drafted the manuscript; and all authors commented on the drafts of the manuscript and approved the final draft of the paper.

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