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Original Article

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

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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*, bla_{OXA30} and bla_{PSE1} genes were estimated at 36.6%%, 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 sakazaii* 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 (P_C), 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), *sul1* gene (sulfonamide), *dfr* gene (dihydroflavonol- 4-reductase/trimethoprim), broad-spectrum b-lactamase and *qacED1* (tetravalent ammonium compounds) (15) and carry various gene cassettes, e.g *aadB*, *dfrA1*, bla_{OXA30} and bla_{PSE1}. 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 LuriaeBertani broth (Merck, Germany) containing 20% glycerol at -80^0 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 (Corrbet, 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
				Denaturation	Annealing	Extension		
intI1	F- TCTCGGGTAACATCAAGG	35	Temp	94 °C	53 °C	72 °C	243	(24)
	R- AGGAGATCCGAAGACCTC		Time	60 Sec	60 Sec	30 Sec		
aadB	F- GGGCGCGTCATGGAGGAGTT	35	Temp	94 °C	55 °C	72 °C	329	(25)
	R- TATCGCGACCTGAAAGCGGC		Time	60 Sec	60 Sec	30 Sec		
dfrA1	F- GGAGTGCCAAAGGTGAACAGC	35	Temp	94 °C	60 °C	72 °C	367	(26)
	R-GAGGCGAAGTCTTGGGTAAAAAC		Time	60 Sec	60 Sec	30 Sec		
bla_{PSE1}	F- GGCAGGCAATCACACTCG	35	Temp	94 °C	50 °C	72 °C	153	(8)
	R-AATCAGGCTCAATACGGTCTAG		Time	30 Sec	60 Sec	60 Sec		
bla_{oxa30}	F-ATTATCTACAGCAGCGCCAGTGCATC	35	Temp	94 °C	50 °C	72 °C	716	(27)
	R-TTCGACCCCAAGTTTCCTGTAAGTGC		Time	30 Sec	60 Sec	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 *bla$_{PSE1}$* genes were estimated at 11 (36.6%) 10 (33.3%), 2 (6.6%) and 0 (0%), respectively (non-significant) (table 2).

Table2: Antibiotic resistance pattern of studied *Enterobacter* isolates according to integron positivity

Antibiotics	Total (n=30)			Integron Positive (n=19)		Integron Negative (n=11)		P<0.05
	R	I	S	R	S	R	S	
	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	
Ampicillin	30(100%)	0(0%)	0(0%)	19(100%)	0(0%)	11(100%)	0(0%)	0.181
Cefepime	28(93.3%)	2(6.66%)	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

Table3: Multi Drugs Resistance profiles

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-CPM-CTX-CIP-GM-AP-IMI-AK	1	
	TM-CPM-CTX-CIP-GM-AP-IMI-NI	2	4
	TM-CPM-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	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*, bla_{OXA30} and bla_{PSE1} 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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References

1. Amin M, Dibachi S, Shahin M. Prevalence of class 1 integrons and plasmid-mediated qnr-genes among Enterobacter isolates obtained from hospitalized patients in Ahvaz, Iran. Le Infez Med 2017; 25: 351-7.
2. Rizk DE, El-Mahdy AM. Emergence of class 1 to 3 integrons among members of Enterobacteriaceae in Egypt. Microb Pathog 2017; 112: 50-6.
3. Sanders W, Sanders CC. Enterobacter spp.: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 1997; 10: 220-41.
4. Davin-Regli A, Pagès JM. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Front Microbiol 2015; 6: 392.
5. Demir T, Baran G, Buyukguclu T, Sezgin FM, Kaymaz H. Pneumonia due to Enterobacter cancerogenus infection. Folia Microbiol 2014; 59: 527-30.
6. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. Mayo Clin Proc 2011; 86: 156-67.
7. Goldstein EJ, Garabedian-Ruffalo SM. Widespread use of fluoroquinolones versus emerging resistance in pneumococci. Clin Infec Dis 2002; 35: 1505-11.
8. Akrami F, Shahandashti EF, Yahyapour Y, et al. Integron types, gene cassettes and antimicrobial resistance profile of Acinetobacter baumannii isolated from BAL samples in Babol, North of Iran. Microb Pathog 2017 ;109: 35-8.

9. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 2008; 153: S347-S57.
10. Molana Z, Ferdosi Shahanndashti E, Gharavi S, et al. Molecular investigation of class I integron in klebsiella pneumoniae isolated from intensive care unit (Shahid Beheshti Hospital of Babol; 2010). J Babol Univ Med Sci 2011; 13: 7-15. [in Persian]
11. Ahangarkani F, Rajabnia R, Shahandashti EF, Bagheri M, Ramez M. Frequency of class 1 integron in Escherichia coli strains isolated from patients with urinary tract infections in North of Iran. Materia Sociomedica 2015; 27: 10-2.
12. Asgharpour F, Rajabnia R, Shahandashti EF, et al. Investigation of class I integron in Salmonella infantis and its association with drug resistance. Jundishapur J Microbiol 20; 7: e10019.
13. Deylam Salehi M, Ferdosi-Shahandashti E, Yahyapour Y, et al. Integron-Mediated Antibiotic Resistance in Acinetobacter baumannii Isolated from Intensive Care Unit Patients, Babol, North of Iran. BioMed Res Int 2017;2017:7157923.
14. Shahandashti EF, Molana Z, Asgharpour F, Mojtahedi A, Rajabnia R. Molecular detection of Integron genes and pattern of antibiotic resistance in Pseudomonas aeruginosa strains isolated from intensive care unit, Shahid Beheshti Hospital, North of Iran. Int J Mol Cell Med 2012; 1: 209-17.
15. Deng Y, Bao X, Ji L, et al. Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob 2015; 14: 45.
16. Mokracka J, Koczura R, Pawłowski K, Kaznowski A. Resistance patterns and integron cassette arrays of Enterobacter cloacae complex strains of human origin. J Med Microbiol 2011; 60: 737-43.
17. Paradowska D, Tomaszewski KA, Balajewicz-Nowak M, et al. Validation of the Polish version of the EORTC QLQ-CX24 module for the assessment of health-related quality of life in women with cervical cancer. Eur J Cancer Care (Engl) 2014; 23: 214-20.
18. Yu WL, Cheng HS, Lin HC, Peng CT, Tsai CH. Outbreak investigation of nosocomial Enterobacter cloacae bacteraemia in a neonatal intensive care unit. Scand J Infect Dis 2000; 32: 293-8.
19. Stoesser N, Sheppard A, Shakya M, et al. Dynamics of MDR Enterobacter cloacae outbreaks in a neonatal unit in Nepal: insights using wider sampling frames and next-generation sequencing. J Antimicrob Chemother 2015; 70: 1008-15.
20. Cantón R, Coque TM, Baquero F. Multi-resistant Gram-negative bacilli: from epidemics to endemics. Curr Opin Infect Dis 2003; 16: 315-25.
21. Molazadeh AR, Gholami MS, Shahi A, et al. Evaluation of antibiotic resistance pattern of isolated gram-negative bacteria from urine culture of hospitalized patients in different wards of Vali-Asr hospital in Fasa during the years 2012 and 2013. J Fasa Univ Med Sci 2014; 4: 275-83.[in Persian]
22. Zheng F, Sun J, Cheng C, Rui Y. Molecular characteristics of carbapenem-resistant gram-negative bacteria in southern China. Microbial Drug Resist 2015; 2: 178-85.
23. Vasilakopoulou A, Psichogiou M, Tzouvelekis L, et al . Prevalence and characterization of class 1 integrons in Escherichia coli of poultry and human origin. Foodborne Pathogens Dis 2009; 6: 1211-8.
24. Leverstein-Van Hall M, Paauw A, Box ATA, et al. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. J Clin Microbiol 2002; 40: 3038-40.
25. Pazhani G, Chakraborty S, Fujihara K, et al. QRDR mutations, efflux system antimicrobial resistance genes in enterotoxigenic Escherichia coli isolated from an outbreak of diarrhoea in Ahmedabad, India. Indian J Med Res 2011; 134: 214-23.
26. Lapierre L, Cornejo J, Borie C, Toro C, San Martín B. Genetic characterization of antibiotic resistance genes linked to class 1 and class 2 integrons in commensal strains of Escherichia coli isolated from poultry and swine. Microb Drug Resist 2008; 14: 265-72.
27. De Vito D, Monno R, Nuccio F, et al. Diffusion and persistence of multidrug resistant Salmonella Typhimurium strains phage type DT120 in southern Italy. BioMed Res Int 2015;2015:265042..