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Prevalence of class 1 integrons and plasmid-mediated *qnr*-genes among *Enterobacter* isolates obtained from hospitalized patients in Ahvaz, Iran

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SUMMARY

Quinolones are frequently used classes of antimicrobials in hospitals, crucial for the treatment of infections caused by Gram-negative bacteria. The inappropriate use of quinolones and other antimicrobial agents for the treatment of bacterial infections leads to a significant increase of resistant isolates. The acquisition of antimicrobial resistance may be related to achievement of resistance determinant genes mediated by plasmids, transposons and gene cassettes in integrons. The objective of this cross-sectional study, conducted from December 2015 to July 2016 at two teaching hospitals in Ahvaz, southern Iran, was to screen for the presence of class 1 integrons and quinolone resistance genes in clinical isolates of *Enterobacter* spp. In all, 152 non-duplicated *Enterobacter* isolates were collected from clinical specimens and identified as *Enterobacter* spp. using standard microbiological methods. Antimicrobial susceptibility test was determined using the disc diffusion method according to the CLSI recommendation. Determination of class 1 integrons and PMQR genes was assessed by PCR. Analysis of antibiotic susceptibility tests showed that the highest antibiotic resistance was toward ciprofloxacin (55.3%), while the lowest level was observed against meropenem (34.9%). Moreover, 47.4% (72/152) and 29% (44/152) of isolates were positive for class 1 integron and quinolone resistance genes, respectively. The relative frequencies of antibiotic resistance were significantly higher among class 1 integron-positive isolates. In summary, our results highlight the importance of PMQR genes in the emergence of quinolone-resistant *Enterobacter* isolates. Moreover, it seems that class 1 integrons have a widespread distribution among *Enterobacter* isolates and have clinical relevance to multiple-drug-resistant isolates.

Keywords: antibiotic resistance, *Enterobacter*, integron, quinolones.

INTRODUCTION

Enterobacter spp. is an ubiquitous Gram-negative bacterium responsible for a broad spectrum of infections such as urinary tract, bloodstream, respiratory tract, intra-abdominal, pneumonia, and soft tissue infections [1, 2]. This organism has emerged as nosocomial pathogen associated with significant morbidity and mortality, especially among hospitalized patients at intensive care units (ICUs) [3]. Quinolones are frequently used classes of antimicrobials in hospital settings, crucial for the treatment of infections caused by Gram-negative bacteria [4, 5]. The widespread and inappropriate use of quinolones and other antimicrobial agents for the treatment of bacterial infections lead to a significant increase of resistant isolates which represent a great public health concern [5, 6]. Several studies reported the high rate of multiple drug resistant (MDR) strains of Enterobacteriaceae in Iranian healthcare settings

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[7-9]. The acquisition of antimicrobial resistance in *Enterobacter* spp. may be related to achievement of resistance determinant genes mediated by plasmids, transposons and gene cassettes in integrons [10, 11]. Resistance to quinolones is often due to several mechanisms such as chromosomal point mutations in topoisomerase IV and DNA gyrase, decreased permeability, and changes in the expression of efflux pumps [12]. Recently, plasmid-mediated quinolone resistance (PMQR) has also been demonstrated in several studies worldwide [13]. To date, several PMQR genes have been identified from clinical isolates of *Enterobacter*, including *qnrA*, *qnrB*, and *qnrS* [12, 13].
Another important mechanism of resistance genes dissemination which has been identified among bacteria is the location of these genes on integrons [14]. These elements are located on plasmids and chromosomes and are divided into different classes based on the amino acid sequences of their integrase genes [14, 15]. Class 1 integron, as the most prevalent class, is predominantly associated with MDR Gram-negative bacteria, as well as *Enterobacter* isolates [15]. The knowledge about trends of antibiotic resistance and mechanisms of resistance is a rational way to reduce the risk of antibiotic treatment failure. Therefore, the objective of this study was to screen for the presence of the class 1 integrons and quinolone resistance genes in clinical isolates of *Enterobacter* spp. from Ahvaz, South of Iran.

METHODS

Study design and setting
This cross-sectional study was conducted in the period December 2015 - July 2016 at two teaching hospitals affiliated with Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. This study was approved by the ethics committee of the Ahvaz Jundishapur University of Medical Sciences. The isolates were obtained from various clinical specimens such as blood, wound, urine, and then were transported to the microbiology laboratory for further analysis. Specimens were cultured onto blood agar and MacConkey's agar. Bacterial isolates were identified as *Enterobacter* spp. using standard microbiological methods including Gram staining and reactions with biochemical tests. The confirmed isolates were stored at -80°C in trypticase soy broth containing 20% glycerol.

Antimicrobial susceptibility testing
Antimicrobial susceptibility tests were determined using disc diffusion method on Mueller- Hinton agar (Himedia, India) according to the CLSI recommendation for levofloxacin (5 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), gatifloxacin (30 µg), imipenem (10 µg) and meropenem (10 µg), (Mast co. UK) [16]. *Escherichia coli* ATCC 25922 strain was used as standard quality control. Intermediate-resistant isolates were accounted as resistant.
Class 1 integrons and quinolone resistance genes assay
Genomic deoxyribonucleic acid (DNA) was extracted from *Enterobacter* spp. isolates by boiling method as described previously [17]. The extracted DNA was dissolved in 100 µl sterile distilled water and stored in -20˚C. Polymerase chain reaction (PCR) amplification was performed to detect the presence of specific *qnrA*, *qnrB* genes and class 1 integrons using the specific primers [18] (Table 1). Amplification products were analyzed using 1.5% agarose gel with KBC power load dye (CinnaGen Co. Iran). Positive results were confirmed by direct sequencing of the PCR products.

Statistical analysis
Data were analyzed using SPSS™ software (IBM corp., USA) version 21.0. The results are presented as descriptive statistics in terms of relative frequency. Chi-square and Fisher's exact tests were used to estimate any statistical association. Statistical significance was regarded as P values <0.05.

RESULTS

In the present study, 152 *Enterobacter* spp. were isolated from different clinical samples of studied hospitals based on phenotypic and genotypic characteristics. Overall, 95 (62.5%) *Enterobacter* spp. isolates were obtained from male and 57 (37.5%) from female samples.
Analysis of antibiotic susceptibility tests showed that the highest antibiotic resistance was toward ciprofloxacin (55.3%); while, the lowest level of antibiotics resistance was observed against meropenem (34.9%). The full results of antibiotic susceptibility assay for *Enterobacter* isolates are shown in Table 2.

The molecular results showed, of 152 isolates, 47.4% (72/152) and 29% (44/152) of isolates were positive for the *int1* and *qnrB* genes, respectively. Meanwhile, 22.4% of the isolates were found positive for both *qnrB* and *int1* genes simultaneously, and none of the isolates were found to be positive for the *qnrA* gene (Figure 1 and 2). In addition, 61.4% (27) *qnr*-positive isolates were obtained from urine samples, followed by wound samples 29.5% (13/44).

The results revealed that 79.5% and 65.9% of clinical isolates of *Enterobacter* carrying the *qnrB* genes

Table 1 - Oligonucleotide sequences of primer sets for PCR.

Primer	*Sequence*	*PCR Product Size (bp)*	*Ref.*
Int1 -F	5′- GCG AAG TCG AGG CAT TTC TGT C -3′	767	[18]
Int1 -R	5′- ATG CGT GTA AAT CAT CGT CGT AGA GA -3′		
qnrA-F	5′- GAT AAA GTT TTT CAG CAA GAG G -3′	543	[18]
qnrA-R	5′- ATC CAG ATC GGC AAA GGT TA -3′		
qnrB-F	5′-GGC ATT GAA ATT CGC CAC TG -3′	263	[18]
qnrB-R	5′-TTT GCT GCT CGC CAG TCG A -3′		
16s rRNA -F	5′- GAT GAC CAG CCA CAC TGG AA -3′	213	[18]
16s rRNA -R	5′- GGA GTT AGC CGG TGC TTC TT -3′		

Table 2 - Antibiotic resistance pattern of studied *Enterobacter* isolates according to integron positivity

Antibiotics	*Total N=152*		*Integron Positive N=72*		*Integron Negative N=80*		*P value*
	R[a] No. (%)	*S[b] No. (%)*	*R No. (%)*	*S No. (%)*	*R No. (%)*	*S No. (%)*	
Ciprofloxacin	84 (55.3)	68 (44.7)	55 (76.4)	17 (23.6)	29 (36.3)	51 (63.8)	<0.001
Levofloxacin	63 (41.4)	89 (58.6)	44 (61.1)	28 (38.9)	19 (23.8)	61 (76.3)	<0.001
Norfloxacin	70 (46.1)	82 (53.9)	49 (68.1)	23 (31.9)	21 (26.3)	59 (73.8)	<0.001
Gatifloxacin	71 (46.7)	81 (53.3)	50 (69.4)	22 (30.6)	21 (26.3)	59 (73.8)	<0.001
Imipenem	80 (52.6)	72 (47.4)	42 (58.3)	30 (41.7)	38 (47.5)	42 (52.5)	0.2
Meropenem	53 (34.9)	99 (65.1)	32 (44.4)	40 (55.6)	21 (26.3)	59 (73.8)	0.03

a= Resistant
b= Susceptible

Table 3 - Antibiotic resistance pattern of studied *Enterobacter* isolates according to *qnr* genes positivity.

Antibiotics	*qnr Positive N=44*		*qnr Negative N=108*		*P value*
	R[a] No. (%)	*S[b] No. (%)*	*R No. (%)*	*S No. (%)*	
Ciprofloxacin	35 (79.5)	9 (20.5)	49 (45.4)	59 (54.6)	<0.001
Levofloxacin	25 (56.8)	19 (43.2)	38 (35.2)	70 (64.8)	0.02
Norfloxacin	29 (65.9)	15 (34.1)	41 (38)	67 (62)	0.002
Gatifloxacin	29 (65.9)	15 (34.1)	42 (38.9)	66 (61.1)	0.004
Imipenem	29 (65.9)	15 (34.1)	51 (47.2)	57 (52.8)	0.01
Meropenem	23 (52.3)	21 (47.7)	30 (27.8)	78 (72.2)	0.005

a= Resistant
b= Susceptible

were resistant to ciprofloxacin and gatifloxacin, respectively (Table 3). Moreover, the relative frequencies of antibiotic resistance, except for imipenem, were significantly higher among class 1 integron-positive isolates compared to negative isolates ($P<0.05$). Also, the presence of *qnrB* gene was significantly associated with higher antibiotic resistance ($P<0.001$).

DISCUSSION

In recent years, *Enterobacter* spp. has been increasingly reported as a cause of nosocomial infections [19, 20]. MDR nature of *Enterobacter* is a major health concern because of high potential for transmission of resistance determinants to other bacterial species. The knowledge about mechanisms of antibiotic resistance has an important role in preventing and overcoming related infections. In the present study, totally 88 (57.9%) isolates were non-susceptible to tested quinolone agents. Out of them, 44 (50%) isolates contained the *qnr* encoding genes, predominantly *qnrB*. The prevalence of PMQR genes among Enterobacteriaceae are varied. Previously, Rezazadeh et al. showed that 2.9% of quinolones non-susceptible *E. coli* isolates harbored *qnrS1*, and none of them contained neither *qnrA* or *qnrB* genes [21]. Peymani and colleagues reported the predominance of *qnrB* genes among quinolone non-susceptible *Klebsiella* isolates, with the prevalence of *qnrB1* 30.6% followed by *qnrB4* 9.7% [22]. In another Iranian multicenter hospital-based survey, 60.3% of *Enterobacter* isolates that were not susceptible to quinolones were positive for *qnr*-encoding genes with a predominance of *qnrB* genes [23]. It seems that closest to our findings, incidence of *qnr* genes among *Enterobacter* isolates compared to other members of Enterobacteriaceae is more common. Regards to these findings, two separate studies from the United States and Jamaica showed a higher prevalence of *qnr*-encoding genes among Enterobacter isolates [24, 25]. Despite of relative agreement on the higher incidence of PMQR genes among *Enterobacter* isolates, the distribution of predominant genes may vary from different regions or even in the same region during different time periods.

As one of the most ubiquitous resistance determinant associated with wide distribution and spread of antimicrobial resistance, class 1 integron has been reported widely among clinical Gram-negative bacteria [15]. Our results showed that 47.4% of investigated *Enterobacter* isolates contained class 1 integron and were significantly associated with higher rates of antibiotic resis-

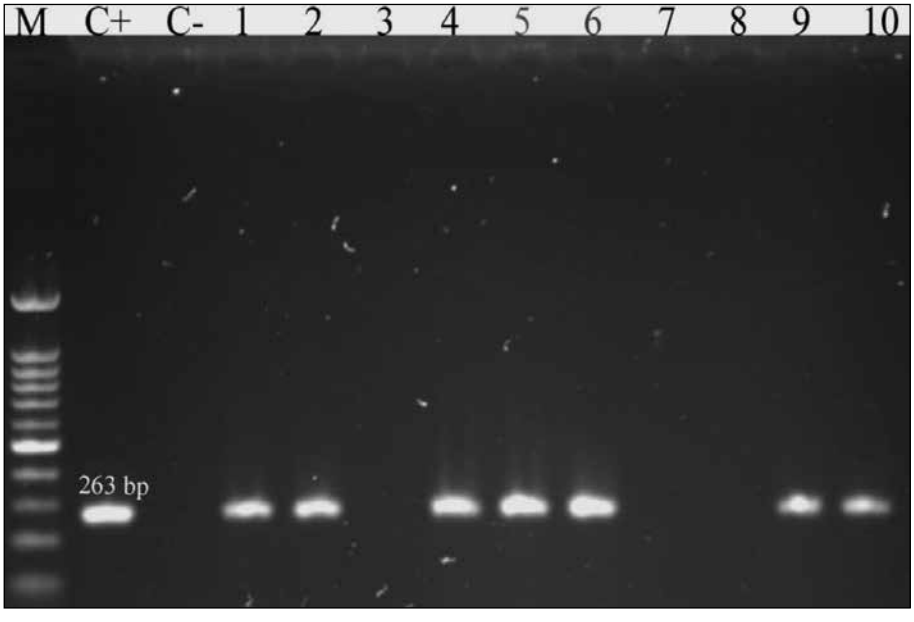

Figure 1 - The amplification results of qnrB genes. Lane M: 100 bp-3k b ladder, lane, 1, 2, 4, 5, 6, 9, 10: positive results for qnrB genes, Lane 3, 7, 8: negative results.

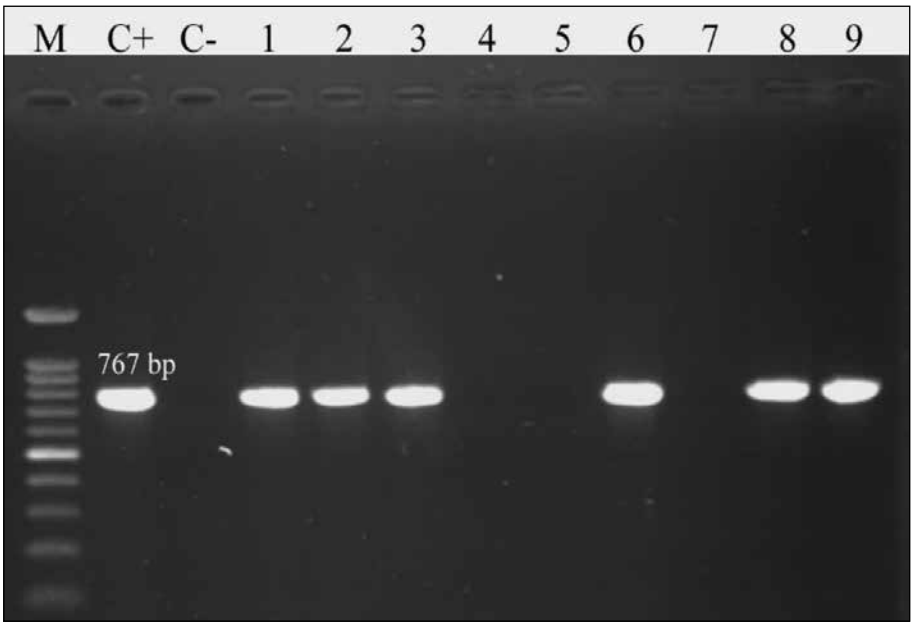

Figure 2 - The amplification results of the Int1 genes. Lane M: 100 bp-3k b ladder, lane 1, 2, 3, 6, 8, 9: positive results for Int1 genes, Lane 4, 5, 7: negative results.

tance. In accordance with our findings, Peymani et al. in a multicenter hospital-based study from North of Iran reported a high incidence of class 1 integron among MDR *Enterobacter cloacae* isolates [26]. Numerous studies reported the prevalence of class 1 integron among clinical Gram-negative bacteria from Iran, and almost all of them reported higher incidence among MDR isolates [27-29]. However, there was some variation according to geographical distribution, type of organisms and source of infections; such finding observed in foreign countries as well [30, 31].

Carbapenems are considered first-line agents for dealing with infections caused by MDR Gram-negative pathogens; however, the emergence of carbapenems-resistant Gram-negative bacteria has become a serious challenge of antimicrobial therapy [4, 32]. In the present study rates of carbapenems resistance were remarkable, especially for imipenem (52.6%). In contrast with our findings, two separate studies from North of Iran showed lower rates of carbapenems resistance among clinical *Enterobacter cloacae* isolates in 2011-2012 [26, 33]. This controversy in our results may arise from an increased prevalence of carbapenem-resistant Enterobacteriaceae due to inappropriate use of antibiotics and empiric therapies in recent years.

There are some limitations related to present study. First, we have not assessed all mechanism of quinolone resistance, since half of quinolone non-susceptible isolates lack any PMQR genes. Second, there is a lack of a typing method, which could clarify the association of clinical isolates.

CONCLUSION

In summary, our results highlight the importance of *qnr*-encoding genes in the emergence of quinolone-resistant *Enterobacter* isolates, since half of quinolone non-susceptible isolates harbor them. Moreover, it seems that class 1 integrons have a widespread distribution among *Enterobacter* isolates and have a clinical relevance with MDR isolates.

Our findings emphasize on continuous surveillance study, and the use of an appropriate infection control policy to prevent and overcome with the emergence and the spread of MDR pathogens in our hospitals.

Conflicts of interest

All authors declare no conflicts of interest

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