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Original Article

Detection of Class 1 Integrons among Gram-negative Bacilli Isolated from Sputum Cultures of Patients with Lower Respiratory Tract Infections in Ahvaz, Iran

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Introduction: Diffusion of antibiotic resistance genes by horizontal gene transfer has led to the fast emergence of multidrug resistance (MDR) among bacteria. Multiple classes of integrons are effective genetic elements which play a significant role in the acquisition and nosocomial dissemination of resistance factors in strains of Gram-negative bacteria, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii*. **Methods:** In this study, 110 sputum samples were collected from hospitalized patients with tract infections. Identification of the isolates was performed by standard biochemical tests. The most frequent Gram-negative isolates were 25 *Enterobacteriaceae* (62.5%), (9 *Enterobacter* spp*,* 11 *Citrobacter* spp*,* and 5 *Escherichia coli*), 6 *P. aeruginosa* (15%) and 9 *Acinetobacter* spp (22.5%). Susceptibility of the isolates to antibiotics was carried out by Kirby-Bauer disk diffusion method according to CLSI guidelines, and finally, the class 1 integrons were detected by PCR. **Results:** Maximum resistance rate among Gram-negative isolates was observed to ceftazidime, co-trimoxazole, and cefotaxime with 89%, 87%, and 82%, respectively. A low-level resistance was recognized for imipenem 32% and gentamicin 34%, while an intermediate level resistance was found against the norfloxacin 40% and ciprofloxacin 44%. Out of 6 *P. aeruginosa* and 9 *A. baumannii* isolates, 2 (33.3%) and 3 isolates (33.3%) were positive for class 1 Integrons, respectively, while all *Enterobacteriaceae* isolates (100%) were negative for class 1 Integrons. Class 1 integrons were detected among of MDR isolates. **Conclusion:** Our results showed that monitoring MDR isolates and detection of class 1 integrons in these isolates is necessary for promotion of antibacterial stewardship. *J Med Microbiol Infec Dis, 2018, 6 (4): 103-107.*

Keywords: Class 1 Integrons, Multidrug Resistance, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacteriaceae.*

INTRODUCTION

Diffusion of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of multidrug resistance (MDR) bacteria [1, 2]. Infections caused by MDR Gram-negative bacteria are known as the primary cause of the enhanced morbidity and mortality among hospitalized patients [1].

Several classes of integrons have been recognized through their distinct *integrase genes* in Gram-negative bacteria. Many antibiotic resistance genes found in Gram-negative bacteria are part of a gene cassette integrated into integrons.

The essential components of an integron include an *intI* gene encoding an integrase, a specific recombination site (*attI*) and a promoter, which promotes the expression of the gene cassettes [2].

Class 1 integrons, as the most prevalent class, is associated with MDR Gram-negative bacteria [3]. Integron-born gene cassettes have been identified mainly in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and members of the *Enterobacteriaceae* family [4]. Various classes of integrons are active genetic elements which play a significant role in the acquisition and nosocomial dissemination of resistance factors in strains of Gram-negative bacteria, *e.g.*, *P. aeruginosa* and *A. baumannii* strains [5, 6]. Class 1 integrons are the most current type present in clinical isolates of the *Enterobacteriaceae* family [7]. *P. aeruginosa* is a crucial pathogen leading to serious infections in hospitals [5].

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This bacteria is also a significant pathogen in immunocompromised patients, such as patients with AIDS, burn wounds, cancer and cystic fibrosis [4]. Acquisition of multidrug resistance by *P. aeruginosa* is becoming a serious concern in many hospitals worldwide [5]. *A. baumannii* is a significant opportunistic pathogen responsible for different types of nosocomial infections. Most of the *A. baumannii* infections are caused by outbreak strains which can spread widely and quickly among patients. Since these strains also display multidrug resistance, it has been suggested that outbreak potency of *A. baumannii* isolates may be related to the presence of integrons [6]. The genes carried by integrons are responsible for resistance to various antibiotics including aminoglycosides, sulphonamides, β-lactams, macrolides, chloramphenicol, antiseptics and disinfectants [4].

This study was aimed to detect class 1 integrons among Gram-negative bacilli isolates originated from sputum cultures of patients with lower respiratory tract infections admitted to university hospitals in Ahvaz, Iran.

MATERIAL AND METHODS

Isolation of bacteria. In present study, 110 sputum samples were collected from patients admitted to university hospitals, affiliated to Ahvaz Jundishapur University of Medical Sciences, Iran, from May 2015 to January 2016. Informed consent was obtained from all adult participants and the parents or legal guardians of minors. The sputum specimens were inoculated onto blood agar, and McConkey agar media. Identification of the isolates was performed by standard bacteriologic tests such as Gram stain, oxidase test, catalase test, and biochemical tests, *e.g.*, TSI, Simmons Citrate agar, Urea, Lysine**,** SIM, MR-VP**,** Gas production and oxidation-fermentation (O/F) [8].

Antimicrobial susceptibility test. Susceptibility test was carried out by Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) (Merck, Germany) according to the 2015 Clinical and Laboratory Standard Institute (CLSI) guidelines. In this test, the concentration of the bacterial isolates was adjusted to 0.5 McFarland, as the standard concentration. The antimicrobial disks included imipenem 10 µg, ceftazidime 30 µg, ciprofloxacin 5 µg, gentamicin 30 µg, cefotaxime 30 µg, norfloxacin 10 µg**,** co-trimoxazole 25 µg provided by a commercial company (Mast Group Ltd., Merseyside, U.K).

The strains *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as controls [9, 10].

DNA Extraction. Genomic DNA was extracted from 6 isolates of *P. aeruginosa*, 9 isolates of *A. baumannii* and 25 isolates of *Enterobacteriaceae* using the boiling method [9]. Briefly, three to four colonies of strains were suspended in 500 µL TE buffer. The samples were incubated at 95°C for 15 min, followed by centrifugation for 10 min at 12000 rpm, 4°C. The supernatants were recovered and stored at -20°C until used. The concentration of the extracted DNA was measured by a photobiometer (Eppendorf, Germany) in 260/280 nm UV long waves [9].

PCR detection of Class 1 Integrons. The 25 µL reaction mixtures contained 1X PCR buffer, 1.5 U/ µl DNA *Taq* polymerase, 0.2 mM dNTPs, 0.4 µM of each forward and reverse primer (Table 1), 1.5 mM $MgCl_2$, 1µL of extracted DNA, and sterile distilled water to the final volume.

PCR amplification of class 1 integrons of *P. aeruginosa* was performed in a thermocycler (Eppendorf, Germany) programmed for an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 59°C for 30 s, and extension at 72°C for 45 s and a final cycle of extension at 72°C for 7 min.

DNA amplification of class 1 integrons for *A. baumannii* was performed in a thermocycler (Eppendorf, Germany) under conditions of initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s, extension at 72°C for 30 s and a final step of extension at 72°C for 5 min.

PCR amplification of class 1 integrons for *Enterobacteriaceae* was performed in a thermocycler (Eppendorf, Germany) under conditions of initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 60 s and a final step of extension at 72°C for 10 min [10].

Two clinical isolates of *P. aeruginosa and A. baumannii* that harbored *intl1* genes were sequenced using automated sequence analyzer (Bioneer, South Korea) and used as positive controls to identify the gene. Distilled water was used as the negative control.

Electrophoresis. The PCR products were resolved on 1.5% agarose gel (SinaClon BioScience Co, Iran) in 1X buffer Tris/ borate/ EDTA buffer (SinaClon BioScience Co, Iran) at 120V for 60 min. The gels were stained with ethidium bromide (SinaClon BioScience Co, Iran) and photographed under a UV gel documentation system (ProteinSimple, San Jose, CA, USA).

Table 1. The primers used for amplification of *intl1* genes of *P. aeruginosa*, *A. baumannii*, and *Enterobacteriaceae* isolates

Gene	Gram-negative bacilli	Primer sequences	Product size (bp)	References
intl1	*P. aeruginosa*	F GCA TCC TCG GTT TTC TGG R GGT GTG GCG GGC TTC GTG	457	[11]
	A. baumanni	F CAGTGGACATAAGCCTGTTC R CCCGAGGCATAGACTGTA	160	[9]
	Enterobacteriaceae	F GTTCGGTCAAGGTTCTGG R CGTAGAGACGTCGGAATG	890	[10]

RESULTS

Based on the bacteriology results, out of 110 specimens, 40 Gram-negative bacteria (36.3%) were isolated by sputum culture. The most frequent Gram-negative isolates were 25 *Enterobacteriaceae* (62.5%), (9 *Enterobacter* spp, 11 *Citrobacter* spp, and 5 *E. coli*), 6 *P. aeruginosa* (15%) and 9 *Acinetobacter* spp (22.5%).

Among the Gram-negative isolates, the maximum resistance rates were observed to ceftazidime, co-trimoxazole, and cefotaxime with 89%, 87%, and 82% respectively. A low-level resistance was recognized for imipenem 32% and gentamicin 34%, while an intermediate level resistance was found against the norfloxacin 40% and ciprofloxacin 44%.

Among the isolates, four *P. aeruginosa* isolates (10%), 7 *A. baumannii* (17%) and 2 *Enterobacteriaceae* (5%) were resistant to imipenem. Antimicrobial agents are shown in Table 2.

Among the 6 *P. aeruginosa* and 9 *A. baumannii* isolates, 2 (33.3%) and 3 isolates (33.3%) were positive for class 1 Integrons, respectively (Figs. 1 and 2), while all *Enterobacteriaceae* isolates (100%) were negative for this gene. The isolates positive for class 1 Integrons were among the MDR isolates.

Table 2. The results of antibiogram test for *P. aeruginosa*, *A. baumannii* and *Enterobacteriaceae*, isolates

Bacteria	Number	Resistant (%)						
		IMP	GM	CP	NOR	CAZ	CTX	SXT
P. aeruginosa	6	10	7	10	10	15	15	15
A. baumannii	9	17	15	22	20	22	22	22
Enterobacteriaceae	25	5	12	12	10	52	45	50
Total	40	32	34	44	40	89	82	87

IMP, imipenem; GM, gentamicin; CP, ciprofloxacin; CAZ, ceftazidime; CTX, cefotaxime; NOR, norfloxacin; SXT, (co-trimoxazole)

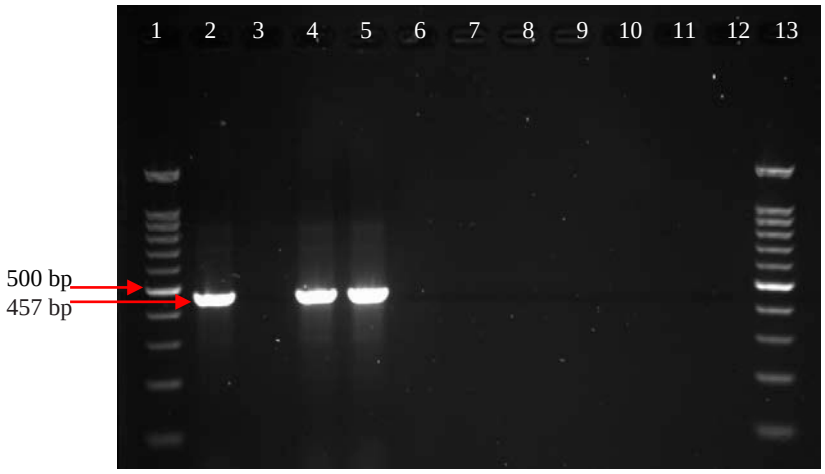

Fig. 1. Amplification of *intl1* gene from *P. aeruginosa* isolates, Lane 1, 100 bp DNA ladder; lane 2, positive control; lane 3, negative control; lanes 4-5, positive clinical samples for *intl1*; lanes 6-12, negative clinical samples; lane 13, 100 bp DNA ladder (SinaClon BioScience Co, Iran)

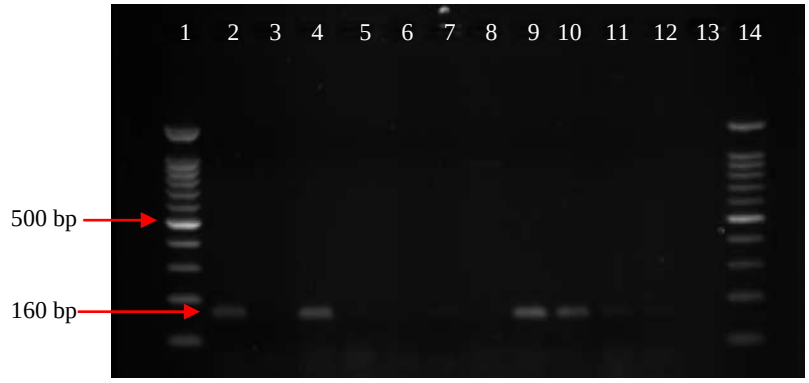

Fig. 2. Amplification of *intl1* gene from *A. baumannii* isolates; Lane 1, 100 bp DNA ladder; lane 2, positive control; lane 3, negative control; lanes 4, 9, 10, positive isolates for *intl1*; lanes 5-8 and 11-13, negative isolates for *intl1*; lane 14, 100 bp DNA ladder

DISCUSSION

Lower respiratory tract infections are a significant cause of morbidity and mortality in hospitalized patients of all age groups. Infections with multidrug-resistant (MDR) Gram-negative bacilli is one of the major causes of more extended hospital stays, increased mortality and costs of hospitalization [12]. *P. aeruginosa*, frequently isolated from clinical specimens, is a critical pathogen that causes various types of infections [4]. Also, *A. baumannii* is an important pathogen causing a variety of nosocomial infections, including ventilator-associated pneumonia, surgical site infections, bacteremia, secondary meningitis, and urinary tract infections [6]. The increasingly rapid spread of multidrug resistance among the *Enterobacteriaceae* is a public health problem [13].

In the present study, we investigated the antibiotic susceptibility pattern and the presence of class 1 integrons in clinical isolates of *P. aeruginosa*, *A. baumannii*, and *Enterobacteriaceae* from patients with lower respiratory tract infections in university hospitals of Ahvaz. The results of the present study indicated considerable levels of antimicrobial resistance among *A. baumannii* and *P. aeruginosa* isolates. Antimicrobial susceptibility test revealed that Gram-negative isolates had the highest resistance against co-trimoxazole, ceftazidime, and cefotaxime. Class 1 integrons were found in 33.3% of *P. aeruginosa* and 33.3% of *A. baumannii*, while none of *Enterobacteriaceae* isolates harbored class 1 integrons. A similar finding in Babol, Iran, has reported *intl1* genes in 39.4% of *P. aeruginosa* strains among which 24.2% were multidrug-resistant, and 15.2% were intermediate or sensitive [14]. Also, another study in Tehran has detected antibiotic resistance and class 1 integrons in clinical isolates of *A. baumannii*. In this study, integron-positive isolates exhibited higher antibiotic resistance rates, and all had MDR phenotype[15]. Nikokar *et al.* (2013) have investigated the antibiotic resistance and prevalence of class 1 integrons among *P. aeruginosa* isolated from burn patients in Guilan, Iran, and showed that 43% of *P. aeruginosa* isolates and 69.2% of multi-drug resistant strains harbored class 1 integrons [16]. Yousef Alikhani *et al.* (2017) have reported the prevalence of class 1 Integrons in clinical and environmental isolates of *P. aeruginosa* and reported class 1 integrons in 57% of the isolates [17]. Gu *et al.* (2007) have reported the prevalence of class 1 Integrons among 40.8% of *P. aeruginosa* and 52.8% of *A. baumannii* isolates from patients in Nanjing, China [18].

In a similar study, Chen *et al.* (2009) have detected class 1 integrons in 38% of *P. aeruginosa* isolates from patients in Zhenjiang, China [19].

In Iran, many studies have reported class 1 integrons in the gram-negative bacteria that were almost all MDR isolates [3, 16]. Similar to other countries, the variations in the prevalence of class 1 integrons among the Gram-negative bacteria might be attributed to geographical areas, source of infections and type of organisms [3].

In conclusion, we demonstrated a high antimicrobial resistance among Gram-negative bacteria isolates in our setting.

The results of this study showed that class 1 integrons occurs among *P. aeruginosa* and *A. baumannii* isolates and seems to play a significant role in multidrug resistance in these bacteria. So, monitoring of drug-resistant isolates along with the presence of class 1 integrons and antibiotic stewardship program is necessary.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Kor SB, Choo QC, Chew CH. New integrand gene arrays from multiversity clinical isolates of members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa* from hospitals in Malaysia. J Med Microbiol. 2013; 62 (3): 412-20.

2. White PA, McIver CJ, Rawlinson WD. Integrons and gene cassettes in the *Enterobacteriaceae*. Antimicrob Agents Chemother. 2001; 45 (9): 2658-61.

3. 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. Infez. Med. 2017; 25 (4):351-7.

4. Yousefi S, Nahaei M, Farajnia S, Ghojazadeh M, Akhi M, Sharifi Y, et al. Class 1 integron and imipenem resistance in clinical isolates of *Pseudomonas aeruginosa*: prevalence and antibiotic susceptibility. Iran J Microbiol. 2010; 2 (3): 113-19.

5. Budak F, Kasap M, Kolayli F, Karadenizli A, Vahaboğlu MH. Integron-associated resistance genes among multidrug-resistant *Pseudomonas aeruginosa* isolated from clinical specimens. Turk J Med Sci. 2012; 42 (1): 149-56.

6. Mohammadi-Barzelighi H, Talebi-Taher M, Adabi M, Javad-Moosavai S, Jabbari M, Rastegar-Lari A. Investigation of Class I, II and III Integrons among *Acinetobacter* strains isolated from ventilator-associated pneumonia patients in intensive care unit of Rasoul Akram hospital in Tehran, Iran. J Med Bacteriol. 2015; 1 (3-4): 1-9.

7. Malek MM, Amer FA, Allam AA, El-Sokkary RH, Gheith T, Arafa MA. Occurrence of classes I and II integrons in *Enterobacteriaceae* collected from Zagazig University Hospitals, Egypt. Front Microbiol. 2015; 6: 601.

8. PM T. Bailey &. Scott's Diagnostic Microbiology. 13th, editor. Elsevier Mosby, St. Louis Missouri, USA2014.

9. Aryanezhad M, Shakibaie MR, Karmostaji A, Shakibaie S. Prevalence of Class 1, 2, and 3 Integrons and Biofilm Formation

in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among ICU and non-ICU Patients. Infect Epidemiol Med. 2016; 2 (4): 1-7.

10. Guo X, Xia R, Han N, Xu H. Genetic diversity analyses of class 1 integrons and their associated antimicrobial resistance genes in *Enterobacteriaceae* strains recovered from aquatic habitats in China. Lett Appl Microbiol. 2011; 52 (6): 667-75.

11. Ohara M, Kouda S, Onodera M, Fujiue Y, Sasaki M, Kohara T, et al. Molecular characterization of Imipenem-resistant *Pseudomonas aeruginosa* in Hiroshima, Japan. Microbiol Immunol. 2007; 51 (3): 271-77.

12. Vishwanath S, Chawla K, Gopinathan A. Multidrug resistant Gram-negative bacilli in lower respiratory tract infections. Iran J Microbiol. 2013; 5 (4): 323-27.

13. Delgado-Valverde M, Sojo-Dorado J, Pascual Á, Rodríguez-Baño J. Clinical management of infections caused by multidrug-resistant *Enterobacteriaceae*. Ther Adv Infect Dis. 2013; 1 (2): 49-69.

14. Rajabnia R, Asgharpour F, Ferdosi Shahandashti E, Khalilian M, Norkhomami S, Shafii M, et al. Class 1 Integron in *Pseudomonas aeruginosa* isolates from different places and devices of ICU in Babol, Iran. Jundishapur J Microbiol. 2013; 6 (2): 138- 43.

15. Mirnejad R, Mostofi S, Masjedian F. Antibiotic resistance and carriage class 1 and 2 integrons in clinical isolates of *Acinetobacter baumannii* from Tehran, Iran. Asian Pac J Trop Biomed. 2013; 3 (2): 140-45.

16. Nikokar I, Tishayar A, Flakiyan Z, Alijani K, Rehana-Banisaeed S, Hossinpour M, et al. Antibiotic resistance and frequency of class 1 integrons among *Pseudomonas aeruginosa*, isolated from burn patients in Guilan, Iran. Iran J Microbiol. 2013; 5 (1): 36-41.

17. Alikhani MY, Parsavash S, Arabestani MR, Hosseini. Prevalence of antibiotic resistance and class 1 Integrons in clinical and environmental isolates of *Pseudomonas aeruginosa.* Avicenna J Clin Microbiol Infect. 2017; 4 (4): e12086.

18. Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, et al. Prevalence and characterization of class I integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. J Clin Microbiol. 2007; 45 (1): 241-43.

19. Chen J, Su Z, Liu Y, Wang S, Dai X, Li Y, et al. Identification and characterization of class 1 integrons among *Pseudomonas aeruginosa* isolates from patients in Zhenjiang, China. Int J Infect Dis. 2009; 13 (6): 717-21.