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Original Article

Prevalence of Class 1, 2, and 3 Integrons and Biofilm Formation in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among ICU and non-ICU Patients

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Background: Infections caused by *Pseudomonas aeruginosa* or *Acinetobacter baumannii* are of greatest concern for hospitalized patients, particularly those in intensive care units (ICUs). The aims of this study were to investigate the prevalence of integrons and biofilm formation among *P. aeruginosa* and *A. baumannii* isolates collected from ICU and non-ICU inpatients.
Materials and Methods: A total of 90 *P. aeruginosa* and 90 *A. baumannii* isolates were recovered from patients admitted into diverse units of Shahid Mohammadi hospital in Bandar Abbas from January to December 2014. Bacterial identification was carried out by phenotypic methods and PCR. Antibiotic susceptibility was measured by disk diffusion assay. The presence of Class 1, 2, and 3 integrons were evaluated by multiplex-PCR. Biofilm quantification was done by microtiter method.
Results: The highest number of isolates (48%) were recovered from ICU patients. About 81% of *P. aeruginosa* isolates were sensitive to piperacillin/tazobactam and ticarcillin, while 60% were resistant to third generation of cephalosporins. In case of *A. baumannii*, all the isolates were sensitive to colistin, but 98% were resistant to other antibiotics ($p \leq 0.05$). Susceptibility to ceftazidime, ticarcillin, imipenem, and piperacillin/tazobactam were higher among isolates obtained from non-ICU patients. Class 1 integron was detected in 13.3% of the *P. aeruginosa* and 40% of the *A. baumannii* isolates, while Class 2 integron was harbored by 7 and 6.6% of the isolates, respectively. Furthermore, 23% of the *A. baumannii* and 12% of the *P. aeruginosa* isolates showed strong biofilm activity.
Conclusion: Class 1 integron-positive isolates were resistant to three classes of antibiotics and predominantly observed in specimens collected from ICU patients showing strong biofilm.

Keywords: ICU, Antibiotic resistance, Integrons, Multiplex-PCR

1. Background

For patientients hospitalized particularly those admitted into ICU,infections with *P. aeruginosa* and *baumannii* are important because these opportunistic pathogens are capable of causing severe invasive infections in patients with cystic fibrosis, neutropenia, iatrogenic immunosuppression, or severe burns (1, 2).. Published data from the National Nosocomial Infections Surveillance (NNIS) System regarding intensive care unit (ICU) patients across the USA show that, in 2003, *Acinetobacter* spp. were responsible for 6.9% of pneumonias, 2.4% of bloodstream infections, 2.1% of surgical site infections, and 1.6% of urinary tract infections, while *Pseudomonas* spp. were responsible for 18.1, 3.4, 9.5, and 16.3% of these infections, respectively (3, 4). High level resistances to third generation of cephalosporins, quinolones, aminoglycosides, tetracycline, and chloramphenicol are widespread in clinical isolates of these two groups of bacteria, and treatment regimens for *A. baumannii* often rely on carbapenems and colistin and for *P. aeruginosa* often rely on piperacillin/tazobactam or ticarcillin (5).

Although heterogeneous studies concluded that acquisition and spread of these microorganisms appeared to be in relation with a large number of variables. Along with the most vital variables were deficiencies in the implementation of infection control guidelines and the use of broad-spectrum antibiotics. The use of carbapenems and third-generation showed to be cephalosporins in relation with the growth of a multidrug resistance (MDR) phenotype by *A. baumannii*, while resistance to carbapenems and fluoroquinolones are implicated in MDR *P. aeruginosa* (6, 7). Several studies showed that integrons are associated with MDR in *P. aeruginosa* and *A. baumannii* isolates (7- 10). They are widely distributed among hospital pathogens and easily transferred to other species by conjugation or transposition. To date, four classes of integrons have been described in gram-negative bacterial isolates. Three main classes of integrons have a 5′ conserved segment, including an *intI* gene encoding an integrase and an *attI* recombination site but have distinct 3′ conserved segments (9). In the Class 1 integrons, the 3′ conserved segment includes three open reading frames (ORFs)-*qacEΔ1*, a deletion derivative of the antiseptic resistance gene *qacE*; the *sul1* sulfonamide resistance gene; and ORF5, of unknown function or *int* genes, as in Tn402 (10, 11). More than 80 different gene cassettes of Class 1 integrons have been described and shown to confer resistance to a wide range of antibiotics such as β-lactams, fluoroquinolones, aminoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin, and antiseptics, and disinfectants (12). The second class of integrons (Class 2) was found in transposon Tn*7* and its derivatives, and its 3′ conserved segment containing five *tns* genes involved in the movements of the transposon, which have been reported most often in isolates within the Enterobacteriaceae family (13). As compared to Class 1 and 2, less is known about Class 3 integron which have been described as rare and found only in a

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limited number of isolates including *Serratia marcescens*, *Klebsiella pneumoniae*, *P. aeruginosa*, *P. putida*, *Alcaligenes xylosoxidans*, and *Delftia* spp (14). Cassettes usually include a single ORF and downstream, an *attC* site which is an imperfect inverted repeat sequence related to a 60bp consensus sequence (14- 16).

There are few reports on the presence of integrons among hospital isolates of *A. baumannii* and *P. aeruginosa* in Iran. In a study conducted in ICU of the Shahid Mohammadi hospital in Bandar Abas south of Iran, 39.4% of the *P. aeruginosa* isolates recovered from different places and devices, carried *intI1* gene (17). No significant differences were seen between the presence of integron and resistance to the antibiotics except for ofloxacin. Similarly, of 63 nonduplicate *A. baumannii* isolates collected from clinical and environmental specimens in the Vali-Asr hospital in the central province of Iran, 98.4% carried a Class 1 integron. The prevalence of Class 2 integron was 15.9% (18).

2. Objectives

The aim of the current study was to evaluate the prevalence of Class 1, 2, 3 integrons and biofilm formation in drug-resistant, colistin susceptible isolates of *P. aeruginosa* and *A. baumannii* in ICU and non-ICU patients of a teaching hospital in south of Iran.

3. Materials and Methods

3.1. Bacterial isolates and identification procedures

A total of 90 isolates of each *A. baumannii* and *P. aeruginosa* were collected from patients hospitalized in different settings of the Shahid Mohammadi hospital in Bandar Abbas (a hospital with an excess of 450 beds) during the Year 2014. This hospital is the main referral treatment center in Hormozgan province, which admits all patients referring from other hospitals across the province. Also, this hospital is the most important center for admission patients with trauma, poisoning, and burn. Demographic information of the patients including length of stay in ICU, underlying diseases, age, gender, pervious surgery, length of stay during index hospitalization (mean) in the ICU and non-ICU inpatients, and previous antibiotic administration were studied. Samples (only one isolate per patient was included in the study) were collected by an expert laboratory technician, inoculated into 5 mL Stuart transport medium (Merck, Darmstadt- Germany), and transferred to the laboratory within 24 hr of the collections. First, Swab specimens were inoculated onto blood agar base with 5% sheep blood and MacConkey agar (Merck, Darmstadt-Germany) and incubated at 37°C for 18–24 hr. *P. aeruginosa* was isolated and identified from patients' cultures by using routine bacteriological procedures including colony morphology, gram staining, pyocyanin pigment production, growth at 44°C, motility, catalase, oxidase tests, and ability to grow in citrimate agar. In case of *A. baumannii*, the isolates were inoculated into cysteine electrolyte deficient (CLED) agar (Hi-Media, India) and incubated at 37°C for 24 hr. Well isolated colonies then were subjected to fermentative/oxidative, motility, oxidase, and other conventional biochemical tests. Final confirmation of *A. baumannii* species was performed by PCR using a set of primer specific for detection of $bla_{\text{OXA-51}}$ gene. Prototype *A. baumannii* ATTC 19606 and *P. aeruginosa* ATCC 27853 were used as quality control strains through this study.

3.2. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed at 37°C on Mueller-Hinton agar containing plates (Merck, Darmstadt-Germany) by using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates according to the Clinical and Laboratory Standards Institute guidelines (CLSI) (19). All antibiotic disks were purchased from Mast Co. Ltd, UK and used as per manufacturer descriptions with an inoculum of 10^4 CFU per spot. Disks zone diameters were interpreted according to the CLSI recommendations for the chosen antibiotics and categorized according to the breakpoints for disk diffusion testing as sensitive, resistant or, intermediate. The following disks were used for antibiotic sensitivity (µg per disc); ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), ticarcillin (TIC, 75 µg), piperacillin/tazobactam (PT, 100 µg), amikacin (AK, 30), gentamicin (GM, 10 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), ciprofloxacin (CP, 5 µg), and doxycycline (D, 30 µg), and colistin (CL, 10 µg). For straightforward result interpretations, intermediate resistant isolates were counted as resistant. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were simultaneously used as antibiotic sensitive control strains.

3.3. Genomic DNA extraction

All specimens were plated onto Luria- Bertani (LB) agar (BioMérieux, Marcy l'Etoile, France) and incubated at 37ºC for 24 hr. Genomic DNA was extracted from 90 isolates of *P. aeruginosa* and 90 isolates of *A. baumannii* by boiling method as described previously (20). Briefly, two well separated colonies of each isolate were dissolved in 200 µl of sterile D/W in 1.5 µl capacity Eppendorf tubes (Eppendorf, Germany) and placed in boiling water bath for 20 minutes. Cell lysates then were centrifuged for 7 minutes at 12.000 rpm, and the supernatants were used for PCR reaction. The quality of the isolated DNA was measured by determination of absorbency at the wavelengths of A260 nm and A280 nm.

3.4. Detection of Class 1, 2 and 3 integrons by multiplex-PCR

All the isolates were screened for integrons Class 1, 2 and 3 by using Multiplex –PCR technique. For detection, we used three sets of primers specific for the integrase *intI1*, *intI2*, and *intI3*. In addition, conserved sequence 5′ (CS) and 3′ (CS) regions of integrons were also amplified. The primers sequences and annealing temperatures used are shown in Table 1. The specificity of primers sequences were confirmed by the BLAST program, available at the NCBI homepage (http://www.ncbi.nlm.nih.gov/BLAST/). The reaction mixture was performed with a final volume of 25 µL. PCR Master Mix was consisted of 3µL 10X PCR buffer, 5µL of extracted DNA (20-50ng), 0.6 µL DNA *Taq* polymerase, 0.5 µL dNTPs Mix (10mM), 1.5 µL (10 pM/µL) of each primer (forward and reverse sequences), 0.8 µL 50 mM $MgCl_2$, and 13.6 µL sterile D/W. DNA amplification was conducted in temperature gradient thermal cycler (Biometra-T300 gradient, Gottingen, Germany) with conditions as follows; initial DNA denaturation at 94^0C for 10 minutes, followed by annealing temperatures of 60^0C (*intI*1), 58^0C (*intI*2), 58°C (*intI3)*, and 52^0C (CS) for 40 seconds, with an extension at 72^0C for 1 minute and a final extension at 62°C for 10 minutes. *K. pneumoniae* ATCC1029 containing the integron Classes 1 and 2 (kindly obtained from Pasteur Institute of Iran) was used as standard control strain.

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Table1. List of primers, sequences, sizes, and annealing temperatures used for the PCR amplification in this study.

Target region	Primer sequence (5′ → 3′)	Size of product	Annealing Temperature °C	References
***intI*-1F** ***intI*-1R**	CAGTGGACATAAGCCTGTTC CCCGAGGCATAGACTGTA	160bp	62	26
***int*I-2F** ***int*I-2R**	GTAGCAAACGAGTGACGAAATG CACGGATATGCGACAAAAAGGT	788bp	62	27
***int*I-3F** ***int*I-3R**	GCCTCCGGCAGCGACTTTCAG ACGGATCTGCCAAACCTGACT	979bp	62	28
***bla*$_{OXA-51}$F** ***bla*$_{OXA-51}$R**	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	353bp	54	20

Abbreviation: bp = base pair, F= forward sequence, R= reverse sequence

3.5. Agarose gel electrophoresis

Agarose electrophoresis was performed on a 1% W/V agarose gel in TBE buffer. The bands were visualized using a transilluminator.

3.6. Biofilm quantification

Biofilm quantification was carried out using crystal violet microtiter method as described previously (21). The isolates divided into strong, moderate, and weak biofilm producers based on cut-off optical density (ODc) of three standard deviations (SDs) above the mean of optical density of the negative control (contained broth only). If OD ≤ ODc, the bacteria were non-adherent; if ODc < OD ≤ 2 × ODc, the bacteria were weakly adherent; if 2 × ODc < OD ≤4 × ODc, the bacteria were moderately adherent; if 4× ODc < OD, the isolates were strongly adherent. The results were performed in triplicate. *P. aeruginosa* PAO1 was considered as positive standard.

3.7. Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL, USA). Prevalence of MDR and integrons were compared using a chi-squared or Fisher's exact test. Differences between the groups were analyzed using independent t-test. A probability value (p- value) within the range of 0.01-0.05 was considered to indicate statistical significance for two-tailed test. For descriptive statistics, we calculated percentage, frequency, mean, median values, and 95% confidence interval (95% CI).

4. Results

4.1. Characteristics of patients

The patients admitted from January to December 2014 in different units of Shahid Mohammadi hospital, Bandar Abbas, were included in this study. The highest number of both *P. aeruginosa* (n= 40) and *A. baumannii* (n= 48) isolates were recovered from ICU patients, 24 isolates from medical ward (MW), and 14 isolates from burn unit, and remaining obtained from other units as shown in Fig. 1. The majority of ICU and burn patients had hospital stay ranged from 10 to 25 days with mean hospital stay of 15±0.2 Most of the patients were aged<35 years. 40% of the patients were female and 60% were male.

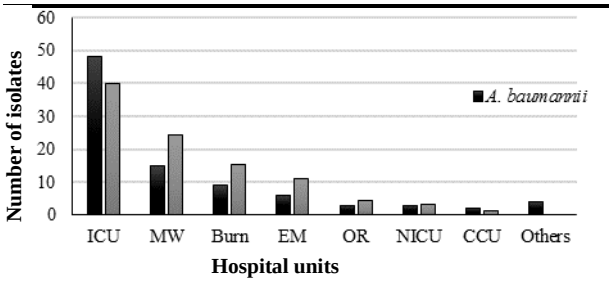

Figure 1. Prevalence of *P. aeruginosa* and *A. baumannii* in different hospital wards of Shahid Mohammadi hospital of Bandar Abbas, Iran. ICU = Intensive care unit, MW=Medical ward, BURN=Burn unit, EM=Emergency, OR=Operating room general surgery, NICU= Neonatal intensive care unit, CCU=Coronary care unit.

4.2. Antimicrobial susceptibility of the isolates

P. aeruginosa isolates were highly susceptible to colistin (100%), piperacillin/tazobactam (81.1%), and ticarcillin (75.5%), while they were resistant to third generation of cephalosporins (with average susceptibility rate of 30%) and doxycycline (48.6%), respectively (Table 2). In case of *A. baumannii*, all the isolates were susceptible to colistin and 23.3% to amikacin, but 98.8% were resistance to other antibiotics tested including imipenem, piperacillin/tazobactam, ticarcillin, cefepime, ceftazidime, ceftriaxone, cefotaxime, and ciprofloxacin. Interestingly, 25.5% of the isolates were sensitive to doxycycline (Table 2). The number of *A. baumannii* isolates exhibiting resistant to different classes of antibiotics, were more as compared to *P. aeruginosa*. (Table 3A). Seven isolates were resistant to more than three classes of antibiotics such as imipenem, piperacillin/tazobactam, cefepime, ceftazidime, ticarcillin, gentamicin, and ciprofloxacin except colistin to which nearly all *Pseudomonas* were sensitive. In this scheme, 13.3% of the isolates were resistant to three or more classes of the antibiotic belonging to the carbapenem groups; penicillins/cephalosporins, aminoglycosides, and quinolone were considered as MDR. The remaining isolates (86.7%) although were resistant to antibiotics but were not classified as MDR. In case of *A. baumannii*, 36 isolates were resistant to all antibiotics including carbapenems, cephalosporins, aminoglycosides, and ciprofloxacin but sensitive to colistin. In this regard, 24 isolates were resistant to 11 antibiotics, while 9 isolates were resistant to 10 antibiotics simultaneously. Antibiotic resistant patterns of the *A. baumannii* isolates are illustrated in Table 3B. In this scheme, 78.8% were considered as MDR. One isolate was found to be extensive drug resistance (XDR). Figure 2 shows the prevalence of MDR strains of *P. aeruginosa* and *A. baumannii* recovered from ICU patients and non-ICU inpatients. The rate of changes in inhibition zone diameter of antibiotics in three different times (months) of collection, January, June, and December 2014, for *A. baumannii* isolates are demonstrated in Fig. 3. The results were expressed as the mean ± SD. There was no significant change in zone diameter during these periods. Similar results were obtained for *P. aeruginosa* isolates (data not shown).

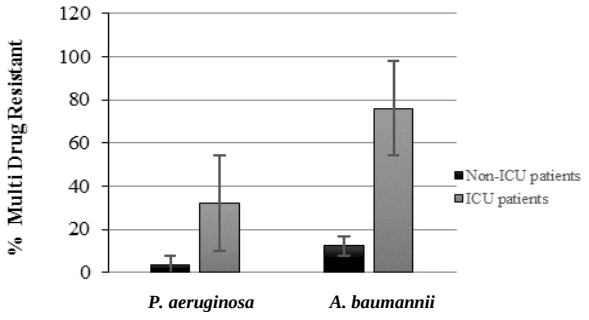

Figure 2. Frequency of multi-drug resistance (MDR) in *P. aeruginosa* and *A. baumannii* isolates recovered from ICU and non-ICU inpatients. The results were expressed as the mean ± SD or as a proportion of the total number of patients or isolates.

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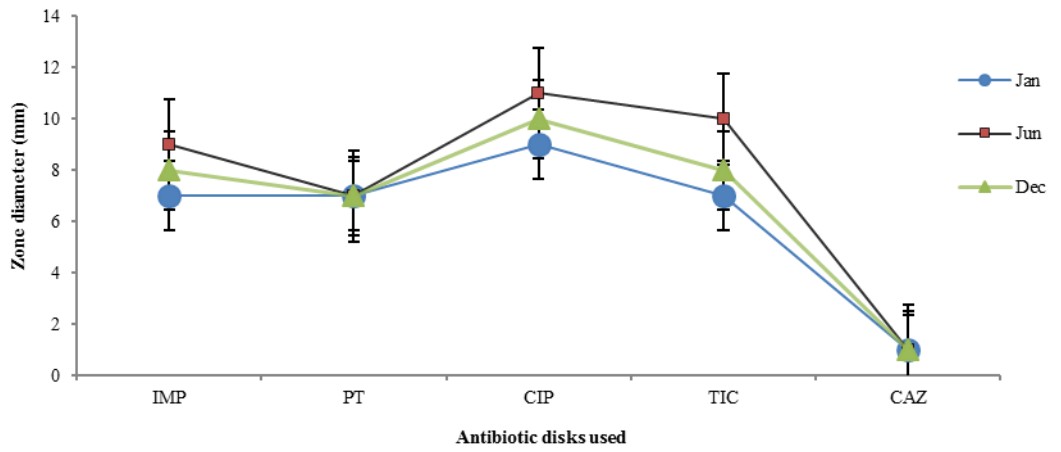

Figure 3. Rate of changes in inhibition zone diameter of antibiotic in three different times (months) of collection. Jan, January; Jun, June; Dec, December 2014.The data are for *A. baumannii* isolates. The results were expressed as the mean ± SD.

Table 2. Antibiotic susceptibility of *P. aeruginosa* and *A. baumannii* isolates recovered from patients in various setting of Mohammadi Bandar Abbas hospital, Iran.

Antibiotic	*P. aeruginosa*			*A. baumannii*		
	R N (%)	I N (%)	S N (%)	R N (%)	I N (%)	S N (%)
IMP	27(30)	3(3.3)	60(66.6)	80(88.8)	7(7.7)	3(3.3)
PT	17(18.8)	0(0.0)	73(81.1)	87(96.6)	2(2.2)	1(1.1)
TIC	16(17.7)	6(6.6)	68(75.5)	88(97.7)	2(2.2)	0(0.0)
FEP	35(38.8)	7(7.7)	48(53.3)	89(98.8)	0(0.0)	1(1.1)
CAZ	44(48.8)	11(12.2)	35(38.8)	89(98.8)	0(0.0)	1(1.1)
CRO	54(60)	16(17.7)	20(22.2)	89(98.8)	0(0.0)	1(1.1)
CTX	48(53.3)	15(16.6)	27(30)	90(100)	0(0.0)	0(0.0)
GM	23(25.5)	9(10)	58(64.4)	79(87.7)	4(4.4)	7(7.7)
AK	21(23.3)	10(11.1)	59(65.5)	65(72.2)	4(4.4)	21(23.3)
CP	22(19.8)	8(7.2)	60(54)	89(98.8)	0(0.0)	1(1.1)
D	54(48.6)	16(17.8)	20(22.2)	60(66.6)	7(7.7)	23(25.5)
CL	0(0.0)	0(0.0)	90(100)	0(0.0)	0(0.0)	90(100)

Abbreviations; IMP, imipenem; PT, piperacillin/tazobactam; TIC, ticarcillin; FEP, cefepime; CAZ, ceftazidime; CRO, ceftriaxone; CTX, cefotaxime; GM, gentamicin; AK, amikacin; CP, ciprofloxacin; D, doxycycline; CL, colistin.

Table 3. Antibiotic resistant patterns, possession of integron classes and hospital unit involved of A) *P. aeruginosa* and B) *A. baumannii* isolates recovered from hospitalized patient in Shahid Mohammadi hospital in Bandar Abbas, south of Iran.

A)

Antibiotic resistance pattern	No. of isolates	No. of antibiotics	Presence of integron classes	Hospital unit	P-value
IMP-PT-FEP-CAZ-TIC-GM-CP	12	7	1	ICU	0.215
IMP -FEP-CAZ-TIC-GM	7	5	1	ICU	0.319
IMP -PT-FEP-CAZ-GM	2	5	1	burn	0.314
IMP -PT-FEP-CAZ-TIC	2	5	-	EM	0.323
IMP -FEP-CAZ-GM	2	4	-	NICU	0.148
FEP-CAZ-TIC-GM	1	4	-	burn	0.314
IMP -CAZ-TIC-GM	1	4	-	OR	0.223
IMP -FEP-CAZ-TIC	1	4	-	CCU	0.321
PT-FEP-TIC-GM	1	4	-	others	0.314

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B)

Antibiotic resistance pattern	No. of isolates	No. of antibiotic	Presence of integron classes	Hospital unit	P-value
IMP-MEM-PT-TC-FEP-CRO-CAZ-CP-D-CTX-GM-AK	36	12	1	ICU	0.221
IMP -MEM-PT-TC-FEP-CRO-CAZ-CP-CTX-GM-AK	24	11	1	ICU	0.358
IMP -MEM-PT-TC-FEP-CRO-CAZ-CP-D-CTX-GM	9	11	1	burn	0.325
MEM-PT-TIC-FEP-CRO-CAZ-CP-D-CTX-GM-AK	1	11	1	NICU	0.311
IMP -MEM-PT-TC-FEP-CRO-CAZ-CP-D-CTX-AK	1	11	1	Burn	0.131
IMP -MEM-PT-TC-FEP-CRO-CAZ-CP-D-CTX	9	10	1	Surgery	0.371
MEM-PT-TC-FEP-CRO-CAZ-CP-CTX-GM-AK	3	10	1	OR	0.200
MEM-PT-TC-FEP-CRO-CAZ-CP-D-CTX-GM	2	10	1	MW	0.300
MEM-PT-TC-FEP-CRO-CAZ-CP-CTX-GM	1	9	1	EM	0.378
MEM-PT-FEP-CRO-CAZ-CP-D-CTX-GM	1	9	-	others	0.300
IMP -MEM-TC-FEP-CRO-CAZ-CP-CTX-GM	1	9	-	surgery	0.172
MEM-FEP-CRO-CAZ-CP-D-CTX-GM	1	8	-	others	0.321

The $\chi 2$ test or Fisher's exact test was used to compare proportions. Statistical significance (p) was calculated using the Pearson Chi-square test in terms of the number of resistant strains and susceptible strains. Intermediate isolates were considered as nonsusceptible isolates. All tests of significance are 2-tailed; p- value was set at range from 0.1 to 0.5.

4.3. Biofilm formation among isolates collected from ICU and non-ICU patients

We studied and compared the number of isolates exhibiting strong biofilm in both *A. baumannii* and *P. aeruginosa* isolates (Fig. 4). Interestingly, we observed that the number of isolates showing strong biofilm in ICU, was higher than non-ICU isolates; moreover, the number of *A. baumannii* isolates forming strong biofilm, was more as compared to *P. aeruginosa* both in ICU and non-ICU patients. The results were repeated at least three times, and similar observations were obtained.

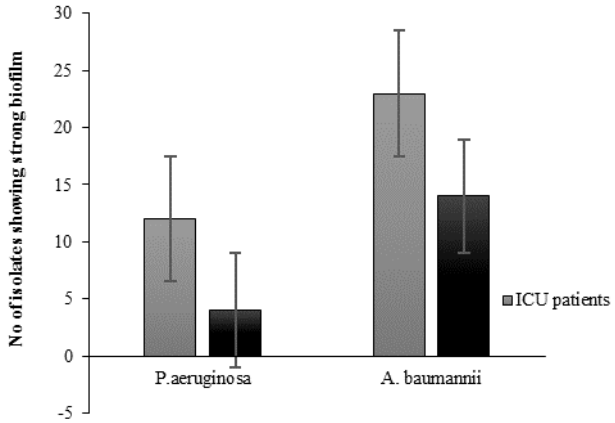

Figure 4. Number of isolates showing positive biofilm collected from patients admitted in ICU and non-ICU. The above results are mean of at least three independent experiments. P≤0.05 was considered statistically significant.

4.4. Prevalence of Class 1, 2 and 3 integrons

Evaluation of integrons classes by multiplex–PCR based method revealed that 13.3 and 7% of the *P. aeruginosa* isolates carried integron Class 1 and 2, while this rate for *A. baumannii* isolates were 40 and 6.6%, respectively. In general, the frequency of the isolates carried Class 1 integron was higher in case of ICU patients as compare with non-ICU inpatients in both cases (Table 3A and B). Integron Class 1 was also detected in isolates obtained from burn patients or those who had surgery. We did not detect integron Class 3 in any of the isolates of both the cases. The agarose gel electrophoresis of PCR products for integrons Classes 1 and 2 in *P. aeruginosa* and *A. baumannii* isolates are illustrated in Fig. 5A and Fig. 5B, respectively. Integron Class 1 positive isolates were statistically more resistant to at least three classes of antibiotics. The chi-square test was used to calculate the *P*-value in terms of resistant, intermediate, and susceptible numbers of integron-positive and integron-negative isolates (2 degrees of freedom). Integrons were significantly associated with resistance to certain antibiotics, including aminoglycosides, quinolones, and beta-lactam agents in both types of isolates. Nevertheless, no correlation was observed between the presence of integron Class 2 and multi-resistance properties of the isolates in both cases (p≤0.05).

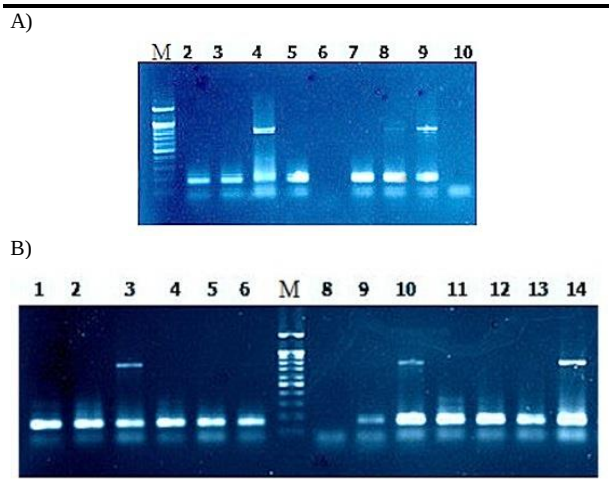

Figure 5. Agarose gel electrophoresis of PCR product of the Class 1 and 2 integrons extracted from A) *P. aeruginosa* and B) *A. baumannii* isolates.

Electrophoresis was conducted in 1% agarose gel and the gel was stained with tracking dye for 5-10 min. The PCR products were then observed under UV. Gel documentation system.

5. Discussion

Recent clinical attention has focused on the increasing frequency of nonlactose fermenting gram-negative pathogens responsible for hospital-acquired infections. In this group, *Acinetobacter* species and *P. aeruginosa* are emerging as pathogens that frequently cause infections in patients with high sever consequences in intensive care units (22). Several investigators have studied various aspects of these infections

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including mechanisms and risk factors of development of resistance as well as the effectiveness and toxicity of various therapeutic options. In multivariate analysis, the strongest risk factors for hospital-acquired infections acquired in ICU were intubation, urinary catheter, severity of illness, involvement of a family member in patient care, and surgery after admission (23).

The dissemination of antibiotic resistance genes between bacteria leads to serious problems in the treatment of infectious diseases. It has been shown that resistance genes can be carried by the integrons. There are limited studies carried out regarding the carriage rate of Class 1 and 2 integrons in *A. baumannii* and *P. aeruginosa* clinical strains in Iran.

In the present study we found that the majority of *P. aeruginosa* isolates were resistant to several antibiotics. Susceptibility to ceftazidime, ticarcillin, imipenem, and piperacillin/tazobactam was higher among isolates from non-ICU inpatients than among isolates from ICU patients. The rate of resistance based on number in population was quite higher in case of *A. baumannii* as compared to *P. aeruginosa* isolates. The majority of *A. baumannii* isolates were resistant to all antibiotic tested except colistin, and at least one isolate was extensive drug resistance. In our study, 70% of the isolates showed pattern of resistance to 12 antibiotics simultaneously, in compare, 7.7% of the *P. aeruginosa* ICU isolates were resistant to six antibiotics, but many were sensitive to piperacillin/tazobactam, imipenem, amikacin, and ticarcillin. The results suggested that colistin could be the drug of choice for treatment of *A. baumannii*, and the reason for susceptibility of all the isolates to colistin may rely on the absence of this antibiotic in routine treatments of inpatients in Shahid Mohammadi hospital. Similarly, piperacillin/tazobactam and ticarcillin antibiotics are used for treatment of neutropenic patients infected with *P. aeruginosa*. In order to see the change in susceptibility of the isolates over the course of time, we compared antibiotic sensitivity tests of bacterial samples in January, June, and December. We did not find any differences in zone diameter in this period of time among the isolates.

In a study, it has been reported that patients with *Escherichia coli* or *Klebsiella* species were more likely to receive inappropriate empiric therapy. In this study, all the ESBL-negative patients were appropriately treated at some point during their antimicrobial course. However, a total of 8% (six out of 75) of the cases failed to receive effective antibiotics at any time during their course of therapy (23). Similar cases may be true for patients infected with *A. baumannii* and *P. aeruginosa* in our case.

In another study carried out in Turkey, a total of 137 strains (77 *A. baumannii* and 60 *P. aeruginosa*) were isolated from various clinical specimens, the highest susceptibility rates were found against colistin (96%) and tigecycline (78%) in *A. baumannii*, and against piperacillin/tazobactam (97%) and piperacillin (93%) in *P. aeruginosa* isolates. The highest resistance rate was determined against piperacillin/tazobactam (95%) in *A. baumannii* strains (24).

Gu *et al.* reported isolation of 98 *P. aeruginosa* isolates and 106 *Acinetobacter* sp. from four general hospitals in the Nanjing area of China. *P. aeruginosa* strains were resistant to antibiotics including cefotaxime, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol. In case of *A. baumannii*, the isolates were resistant to piperacillin, cefotaxime, ceftazidime, cefepime, aztreonam, norfloxacin, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol (25).

We also studied the presence of three different integrons among hospital isolates of *P. aeruginosa* and *A. baumannii*. Interestingly, the prevalence of integron Class 1 in both microorganisms was higher as compared to integron Class 2. Moreover, there was a significant correlation between resistance patterns of the isolates and the presence of integron Class 1among ICU patients as compare with non-ICU patients. The results suggest that resistance to third generation of cephalosporins, carbapenems, aminoglycosides, and aminopenicillins may be resided, as gene cassettes, on integron Class 1, and clonal spread of these resistance genes among patient populations in different hospital setting, particularly in ICU, may also be the reason for MDR phenotypes. We also found that as length of stay in ICU increases for per patient, there is a higher chance to be colonized with MDR isolates of *A. baumannii* or *P. aeruginosa* carrying integron Class 1. In this regard, we found the 13% increase in colonization by *A. baumannii* in patients with length of stay more than 15 days. In addition, biofilm formation assay showed considerable number of both *A. baumannii* and *P. aeruginosa* isolates exhibiting strong biofilm from specimen collected from ICU patients than non-ICU inpatients. This indicates that ICU isolates were more virulent that non-ICU isolates since biofilm formation is associated with increase in virulence capacity of the organism as well. The results also suggest that antibiotic resistance in non-integron-encoding isolates may probably be due to biofilm formation.

A total of 189 *A. baumannii* isolates were collected in 2011 from a teaching hospital in Chongqing, China (26). Among which, 144 isolates (76.2%) were found to have the integron cassette, which was absent in the rest. Among the integron-positive isolates, 136 of the isolates had a Class 1 integron cassette, and eight had a Class 2 integron cassette. None of the isolates was found to encode Class 3 integrons. In another study, Nikokar et al. (27) reported that 37 (43%) *P. aeruginosa* isolates and 27 (69.2%) MDR strains harbored Class 1 integron in a burn center.

6. Conclusion

Acquisition of antibiotic-resistant ICU bacteria depends on ICU-related variables (nurse-to-patient ratios, compliance with hand hygiene) and patient-related factors (severity of illness, prior hospitalization, invasive procedures, antibiotic use). Present investigation showed the emergence of MDR among *A. baumannii* isolates in different units of hospital. The existence of Class 1 and 2 integrons might be responsible for spread and dissemination of antibiotic resistance genes among bacterial population, especially in ICU. Our findings also highlight the emerging challenges that *Acinetobacter* species pose to health care facilities. Further research must be carried out for molecular typing of these isolates.

Conflict of Interests

The authors declare that they have no competing interests in this investigation.

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Authors' Contribution

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