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Characterization of integrons and associated gene cassettes in *Acinetobacter baumannii* strains isolated from intensive care unit in Tehran, Iran

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ABSTRACT

Objective: To determine the antimicrobial susceptibility patterns, the frequency of integrons and associated gene cassettes in *Acinetobacter baumannii* (*A. baumannii*) strains isolated from selected hospital intensive care units.

Methods: During a ten-month period, 120 *A. baumannii* isolates were studied. The resistance rates to different classes of antimicrobial agents were determined. PCR was used to detect different types of integrons and associated gene cassettes.

Results: The resistance rates to the majority of antibiotics tested were found to be between 39.3% and 99.1%. No isolate was observed to be resistant to colistin and polymyxin B. The rate of extensive drug-resistance among these clinical isolates was 62.5%. The prevalence of class 1 and 2 integrons was found to be 74.1% and 12.5%, respectively. Seven different gene cassettes (*ampC*, *aacA4-catB8*, *ISAba1-bla*$_{OXA-23}$*-GES-14*, *aadA2-cm1A6-GES-14-qacF*, *VIM-25-GES-24-qacF*, *dfrA5-ISAba1-bla*$_{OXA-51}$*-bla*$_{OXA-40}$ and *aadA2-GES-11-IMP-1*) were observed in Class 1 integron-carrying strains. Three gene cassettes (*IMP-4*, *VIM-2-VEB-aacA4* and *dfrA2-sat-2-aadA4*) were detected in class 2 integron-bearing *A. baumannii* strains.

Conclusions: A high prevalence of integron was described among multidrug resistant *A. baumannii* in the hospital. The findings highlighted the need for continuous surveillance in order to prevent dissemination of multidrug resistance among *A. baumannii* strains in Iran.

1. Introduction

Acinetobacter baumannii (*A. baumannii*), as an important nosocomial pathogen especially in intensive care units (ICUs), is responsible for a wide range of infections that can be ranged from urinary tract infections to surgical wounds infection, ventilator-associated pneumonia, meningitis, bacteremia, and life threatening infections. The most important factor contributing to the successful extensive distribution of this nosocomial pathogen is stated to be its remarkable ability for the acquisition of a wide variety of antibiotic resistance genes and also adaptation in various harsh environments[1]. The acquisition of a wide variety of antibiotic resistance genes not only leads to an increase in economic burden, but also causes serious therapeutic problems. Moreover, it can lead to difficulties in infection control in hospitals and eradication of the bacteria. The emergence and extremely rapid spread of multidrug resistant *A. baumannii* isolates are becoming a serious concern in global public health. The spread of the resistant genes in

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hospitals and community is mediated by horizontal gene transfer[2]. Mobile elements including plasmids, transposons, or integrons are the most efficient genetic elements promoting acquisition and dissemination of resistance determinants[3]. During the past several decades, despite introduction of new therapeutic options, *A. baumannii* strains have shown a remarkable ability to rapidly develop multidrug resistance (MDR). This rapid increase of MDR is not only due to the intrinsic resistant genes carried by these strains, but also due to their outstanding capacity to acquire resistant elements from other bacteria. Recently, the role of efflux pumps, class B β-lactamase (metallo-beta-lactamase), chromosomal class C β-lactamase AmpC, class D β-lactamase (OXA-type carbapenemase), integrons, and associated insertion sequence elements in the occurrence of MDR has been well documented[3,4]. The acquisition and dissemination of antimicrobial resistant determinants in multidrug resistant *A. baumannii* strains are frequently mediated by integrons.

Integrons are a key element in spreading MDR particularly in Gram-negative pathogens[5]. They are normally motionless but can be transferred through mobile genetic elements, *e.g.* plasmids and transposons. Integrons are genetic elements composed of an *intI* gene encoding an integrase, flanked by a recombination site *attI* and a strong promoter gene, where mobile gene cassettes, mostly containing antibiotic resistance determinants, can be inserted or excised by a site-specific recombination mechanism catalyzed by the integrase. Integrons carry divergent gene cassettes that are rearranged under antibiotic selective pressure[3,5]. In *A. baumannii*, these gene cassettes often contain efflux pump genes, beta-lactam resistance genes, and aminoglycosides resistance genes. To date, several classes of integrons have been distinguished upon the basis of the sequence of the integrase gene. Among the several classes of integrons, class 1 integron is the most prevalent class type that is essential in the emergence and spread of resistance genes, followed by class 2. Reports on the other classes of integrons are scarce[5,6]. According to the literature, class 1 integrons and the pool of associated gene cassettes are the major contributors to the MDR of *A. baumannii* and also could be a useful tool for studying molecular epidemiology in possible cross-infection cases, especially in critical wards of hospitals, such as ICU[2,7]. Although the presence of integrons has already been documented in *A. baumannii* clinical isolates, the content of their cassette has not been fully characterized. The current study was carried out in order to characterize the occurrence of drug resistance, presence and dissemination of different classes of integrons, and associated gene cassettes among *A. baumannii* isolates recovered from inpatients in ICUs.

2. Materials and methods

2.1. Sampling and data collection

The present cross-sectional study was conducted during February–November 2015. During this period, a total of 120 non-repetitive *A. baumannii* isolates were recovered from 490 clinical specimens of hospitalized patients in ICU wards of four hospitals in Tehran, Iran. Duplicate isolates from the same patients were excluded from the study. The study protocol was performed according to the Helsinki declaration and approved by Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (No. 13478). Written informed consent was obtained from the patients to use their samples for research purposes. All the obtained samples were transported to laboratory within 4 h of collection and were processed immediately. Bacterial identification was performed using the conventional biochemical tests and the API 20 NE system (bioMérieux SA, Marcy-1′Etoile, France). *A. baumannii* isolates were stored in tryptic soy broth (Merck Co., Germany) containing 20% glycerol at −70 °C and were subjected to further molecular analysis.

2.2. Antimicrobial susceptibility testing

In vitro susceptibility test was performed using a panel of 17 antibiotics for all the isolates by micro-broth dilution method. The susceptibility test was performed according to the guidelines of the Clinical and Laboratory Standards Institute[8]. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of each antimicrobial agent inhibiting visible growth of the tested isolate. The antimicrobial agents used in the present survey included: amikacin, ampicilin/sulbactam, cefepime, cefotaxim, ceftazidime, ceftriaxone, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, netilmicin, piperacillin/tazobactam, polymixin B, tetracyclin, tobramycin and trimethoprim-sulfamethoxazole. MDR was defined as resistance to three or more unique antimicrobial drug classes[2]. Extensive drug-resistant *A. baumannii* was defined as resistant to three or more unique antimicrobial drug classes and carbapenems[9]. All the antibiotic powders used in the current study were supplied by Sigma–Aldrich (St. Louis, MO, USA). The standard reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains in every test run. The ranges of MIC value used for antimicrobial agents were as follows: 0.5–256 μg/mL of amikacin; 1–128 μg/mL of cefepime; 0.5–256 μg/mL of cefotaxim; 0.25–128 μg/mL of ceftazidime; 2–256 μg/mL of ceftriaxone; 0.125–32 μg/mL of ciprofloxacin; 0.125–4 μg/mL of colistin; 0.25–64 μg/mL of gentamicin; 0.125–256 μg/mL of imipenem; 0.125–128 μg/mL of meropenem; 0.5–64 μg/mL of netilmicin; 0.125–4 μg/mL of polymixin B; 0.5–64 μg/mL of tetracycline; 0.5–64 μg/mL of tobramycin; 4–256 μg/mL of piperacillin/tazobactam; 4–512 μg/mL of ampicilin/sulbactam and 2–128 μg/mL of trimetoprim-sulfamethoxazole.

2.3. Extraction of plasmid and genomic DNA

Genomic DNA of strains was extracted using the commercial kit (InstaGene Matrix, Bio-Rad, Hercules, CA, USA). The QIAGEN Plasmid Midi Kit was used for plasmid DNA extraction according to the manufacturer's instruction.

2.4. Integron assessment in A. baumannii isolates

The existence of integrons was confirmed using PCR with degenerate primers described by Moura *et al.*[10]. PCR conditions for amplification of the int1 and the int2 by thermocycler (Eppendorf Co., Hamburg, Germany) are as follows: initial denaturation for 4 min at 94 °C, 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 50 s, and extension at 72 °C for 1 min. The final extension was carried out at 72 °C for 3 min.

2.5. Mapping of the integrons

Amplification of the variable region in integron was performed using primer pairs introduced by Moura *et al.*[10]. Integron cassette PCR products were purified by the QIAquick Gel Extraction Kit (Qiagen Co., Hilden, Germany). Purified PCR products were subjected to sequencing with an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin–Elmer Co., Foster City, CA, USA) in both directions. The sequences were assembled making use of SeqMan program within the Lasergene suite version 7 (DNASTAR Inc., Madison, WI, USA). Sequences obtained were compared with those in the NCBI database using a BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the integron database INTEGRALL (http://integrall.bio.ua.pt/).

2.6. Statistical analysis

Statistical analysis was carried out using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). *Chi*-square test was run to determine the *P* value. A *P* value less than 0.05 was considered as statistically significant.

3. Results

3.1. Bacterial strains

During the ten-month period of the study, a total of 120 *A. baumannii* clinical isolates were recovered from 490 clinical specimens of hospitalized patients in ICU wards in four hospitals in Tehran, Iran. *A. baumannii* isolates were obtained from different clinical specimens including respiratory secretions (n = 42, 35%) followed by blood (n = 30, 25%), wound (n = 21, 17.5%), urine (n = 11, 9.2%), catheter (n = 8, 6.7%), cerebrospinal fluid (n = 5, 4.2%), ascitic fluid (n = 2, 1.7%) and pleural effusion (n = 1, 0.7%). The average age was 41 (median 40.8, ranging from 4 months to 63 years of age). In the present study, 67.5% of the patients were men and 32.5% were women.

3.2. Antimicrobial resistance profile

The result of antimicrobial susceptibility test of 120 *A. baumannii* clinical isolates showed that the majority of isolates were resistant to imipenem (99.1%), ceftriaxone (92.8%), ciprofloxacin (89.3%) and amikacin (80.3%). The lowest levels of resistance were found to be related to netilmicin (39.3%). All the isolates were susceptible to colistin and polymixin B. *In vitro* susceptibility of the *A. baumannii* isolates to 17 antibiotics was tested. The ranges of MIC_{50} and MIC_{90} are summarized in Table 1. Antibiogram showed that 90% of the isolates were inhibited by 1 μg/mL of colistin. The data showed that 50% of the isolates were inhibited by 0.5 μg/mL of polymixin B. One hundred (83.3%) isolates were inhibited by concentration of polymixin B that did not exceed 1 μg/mL. All the isolates were MDR while, out of 120 isolates tested, 75 (62.5%) were extensive drug-resistant. Resistance profile pattern showed that 10 isolates (8.3%) were resistant to 15 antibiotics, 15 isolates (12.5%) were resistant to 14 antibiotics, 11 isolates (9.2%) were resistant to 13 antibiotics, 21 isolates (17.5%) were resistant to 12 antibiotics, 17 isolates (14.2%) were resistant to 10 antibiotics, 8 isolates (6.7%) were resistant to 8 antibiotics, 15 isolates (12.5%) were resistant to 7 antibiotics, 13 isolates (10.8%) were resistant to 6 antibiotics, and 10 isolates (8.3%) were resistant to 5 antibiotics. The predominant multiple resistance profile among the isolates studied was resistance to 12 antibiotics (amikacin, cefepime, cefotaxim, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, meropenem, netilmicin, tetracyclin, and tobramycin) which were common among 16 (13.3%) isolates.

3.3. Integron assessment and sequencing of cassette arrays

The results of the study showed that integrons were widely distributed among *A. baumannii* clinical isolates (93.3%). Class 1 integrons were detected in 89 (74.1%) of the 120 isolates while class 2 integrons were detected only in 15 (12.5%) isolates. Class 3 integron was not detected among the isolates. Among

Table 1
Antibiotic resistance pattern and integron frequency of 120 *A. baumannii* isolated from hospitalized patients in ICU.

Antibiotics	Integron positive (n = 112)			Inetgron negative (n = 8)			MIC (μg/mL)		
	R [n (%)]	I [n (%)]	S [n (%)]	R [n (%)]	I [n (%)]	S [n (%)]	Range	50%	90%
Ampicillin/sulbactam	61 (54.5)	2 (1.8)	49 (43.7)	3 (37.5)	0 (0.0)	5 (62.5)	4.000–512.000	326.0	642
Piperacillin/tazobactam	85 (75.9)	2 (1.8)	25 (22.3)	5 (62.5)	1 (12.5)	2 (25.0)	4.000–256.000	128.0	256
Cefepime	65 (58.0)	3 (2.7)	44 (39.3)	4 (50.0)	2 (25.0)	2 (25.0)	1.000–128.000	64.0	64
Cefotaxime	81 (72.3)	12 (10.7)	19 (17.0)	7 (87.5)	0 (0.0)	1 (12.5)	0.500–256.000	128.0	128
Ceftazidime	55 (49.1)	0 (0.0)	57 (50.9)	3 (37.5)	2 (25.0)	3 (37.5)	0.250–128.000	32.0	64
Ceftriaxone	104 (92.8)	1 (0.9)	7 (6.3)	6 (75.0)	1 (12.5)	1 (12.5)	2.000–256.000	64.0	128
Imipenem	111 (99.1)	0 (0.0)	1 (0.9)	1 (12.5)	0 (0.0)	7 (87.5)	0.125–256.000	32.0	64
Meropenem	70 (62.5)	1 (0.9)	41 (36.6)	5 (62.5)	2 (25.0)	1 (12.5)	0.125–128.000	16.0	32
Gentamicin	89 (79.5)	8 (7.1)	15 (13.4)	6 (75.0)	0 (0.0)	2 (25.0)	0.250–64.000	32.0	64
Amikacin	90 (80.3)	5 (4.5)	17 (15.2)	0 (0.0)	3 (37.5)	5 (62.5)	0.500–256.000	64.0	64
Netilmicin	44 (39.3)	1 (0.9)	67 (59.8)	4 (50.0)	0 (0.0)	4 (50.0)	0.500–64.000	32.0	32
Tobramycin	61 (54.5)	2 (1.8)	49 (43.7)	4 (50.0)	2 (25.0)	2 (25.0)	0.500–64.000	16.0	32
Tetracyclin	75 (67.0)	2 (1.8)	35 (31.2)	2 (25.0)	1 (12.5)	5 (62.5)	0.500–64.000	32.0	64
Polymixin B	0 (0.0)	0 (0.0)	112 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0.125–8.000	0.5	1
Colistin	0 (0.0)	0 (0.0)	112 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	0.125–4.000	1.0	1
Ciprofloxacin	100 (89.3)	5 (4.5)	7 (6.2)	5 (62.5)	0 (0.0)	3 (37.5)	0.125–32.000	16.0	32
Trimetoprim-sulfamethoxazole	83 (74.1)	0 (0.0)	29 (25.9)	5 (62.5)	0 (0.0)	3 (37.5)	2.000–128.000	76.0	152

R: Resistant; I: Intermediate; S: Sensitive.

the 120 investigated isolates, 8 isolates (6.7%) were found to simultaneously carry class 1 and 2 integrons. The frequency of the integron among 120 *A. baumannii* isolated from ICU wards is presented in Table 1. In 89 class 1 integron-carrying isolates, seven different gene cassettes were observed. The seven types of class 1 integron cassettes included *ampC*, *aacA4-catB8*, *ISAba1-bla$_{OXA-23}$-GES-14*, *aadA2-cm1A6-GES-14-qacF*, *VIM-25-GES-24-qacF*, *dfrA5-ISAba1-bla$_{OXA-51}$-bla$_{OXA-40}$*, and *aadA2-GES-11-IMP-1*. Three gene cassettes (*IMP-4*, *VIM-2-VEB-aacA4* and *dfrA2-sat-2-aadA4*) were detected in 15 (12.5%) class 2 integron-bearing *A. baumannii* strains. Out of 89 isolates carrying class 1 integrons, 66 (74.2%) were located on chromosome and 23 (25.8%) on plasmid. Out of 15 isolates carrying class 2 integrons, 12 (80%) were located on plasmid and 3 (20%) on chromosome. Information about gene cassette arrays found in integron positive isolates is shown in Table 2. The numbers of cassette genes in class 2 integrons were much more limited compared with those of class 1 integrons. The most prevalent type of gene cassette in class 1 integron-bearing *A. baumannii* isolates was *aacA4-catB8* cassette, accounting for 27% of class 1 integron cassettes. In isolates harboring both class 1 and 2 integrons simultaneously, gene cassettes *GES-11-IMP-4-VIM-2* and *dfrA11-aacA4-bla$_{OXA}$* were detected.

Table 2
Various cassette arrays found in class 1 and class 2 integrons.

Integron class	Frequency (%)	Gene cassette arrays [*n* (%)]	Location
Class 1 integron	89 (74.1)	*ampC* [22 (24.7)]	Chromosome
		aacA4-catB8 [24 (27)]	Chromosome
		ISAba1-bla$_{OXA-23}$-GES-14 [8 (9)]	Chromosome
		aadA2-cm1A6-GES-14-qacF [10 (11.2)]	Chromosome
		VIM-25-GES-24-qacF [9 (10.1)]	Plasmid
		dfrA5-ISAba1-bla$_{OXA-51}$-bla$_{OXA-40}$ [2 (2.2)]	Chromosome
		aadB-GES-11-IMP-1 [14 (15.8)]	Plasmid
Class 2 integron	15 (12.5)	*IMP-4* [7 (46.7)]	Plasmid
		VIM-2-VEB-aacA4 [5 (33.3)]	Plasmid
		dfrA2-sat2-aadA4 [3 (20)]	Chromosome
Class 1 and 2 integron	8 (6.7)	*GES-11-IMP-4-VIM-2* [1 (12.5)]	Plasmid
		dfrA11 [2 (25)]	Chromosome
		aacA4-bla$_{OXA-58}$ [5 (62.5)]	Chromosome
Class 3 integron	0 (0.0)		
Without integron	8 (6.7)		
Total	120 (100.0)		

3.4. Nucleotide sequence accession numbers

The nucleotide sequences of cassette arrays obtained in this study are available in the DNA data bank of Japan under accession numbers LC107421, LC107422, LC107423, LC107424, LC107425, LC107426 and LC107606.

4. Discussion

During the recent years, high incidence of multidrug resistant *A. baumannii* has become a serious threat worldwide[1]. High resistance to different classes of antimicrobial agents in *A. baumannii* clinical isolates contributes to its persistence in the hospitals and health care settings[1,9]. Dissemination of the antimicrobial resistance genes through integrons in *A. baumannii* is now a worrying evolution[3]. The most predominant mechanism in the distribution of antimicrobial resistance among microbial populations is horizontal gene transfer; indeed during the past decade, it has received the most attention among all the multidrug resistant *A. baumannii* strains[2,3,9]. Also, the role of integrons as a vital system in horizontal transfer of antibiotic resistance has been well documented[11,12]. As previously mentioned, integrons are linked to MDR and subsequently constrict the therapeutic options and worsen clinical outcomes. In the present study, the main source of *A. baumannii* isolates was respiratory specimen (35%). This is in accordance with the data presented by other studies[13]. Overall, the resistance rates were high to most antimicrobial drugs. Since carbapenems have been used as a drug of choice for the treatment of serious nosocomial infections caused by *Acinetobacter*, carbapenem-resistant *A. baumannii* strains have been reported worldwide[14]. The present study demonstrated high level of resistance to carbapenems (imipenem 99.1% and meropenem 62.5%). The resistance rate to imipenem in the present survey was higher than that reported for Turkey (80%)[15], Iran (53%)[16], Nepal (36%)[17], Russia (45%)[18], Poland (41%)[19], China (72.2%)[20] and Taiwan (36.6%)[21]. The high resistance rate to imipenem in our study could be attributed to the improper prescription of this antibiotic in clinics, extensive misuse of carbapenems, and production of class D carbapenem hydrolyzing enzymes OXA-β-lactamases and class B metallo β lactamases (MBLs) as important contributors to carbapenem resistance in *A. baumannii*. In the present study, colistin and polymyxin B exhibited a potent activity against *A. baumannii* isolates, which is in accordance with the findings reported in the recent studies in China[22], Iran[23], and USA[24]. High susceptibility rate to these antibiotics could be attributed to limited use of these antibiotics in life threatening conditions due to its serious side effects. The data obtained in the present study revealed an increase (> 50%) of resistance in *A. baumannii* isolates to beta lactams. This finding is consistent with those reported in the recent studies from Iran[25], China[20], Turkey[15] and Poland[19]. Production of β-lactamases, changes in penicillin-binding proteins, alterations in the structure of porin proteins and efflux pumps are the main mechanisms of resistance to beta lactams[14,24]. Comparison of the findings with those of the other studies reveals that resistance to ciprofloxacin among *A. baumannii* isolates is increasing[19–21]. However, this finding is in contrast to a recent report by Zhu *et al.* from China[22]. Among *A. baumannii* strains, the lowest resistance rate (with the exception of polymixin B and colistin) was noted for netilmicin. Overall, the findings of the current

study are consistent with those reported in the recent studies from Poland demonstrating the lowest resistance rate to netilmicin in comparison with the other tested antibiotics[19]. Screening for MDR frequency among *A. baumannii* isolates showed an alarming trend of resistance increase to multiple antibiotics. Several studies have also shown emergence of MDR strains and it is likely due to improper use of antimicrobial agents, which limits therapeutic protocols[1,15]. The MDR frequency among tested isolates is similar to that stated previously in Poland (100%)[19] and China (93.5%)[13], and is considerably higher than those reported in other recent reports from Thailand (21.1%)[26] and China (61.3%)[27].

The high prevalence of class 1 integrons among multidrug resistant *A. baumannii* clinical isolates has been confirmed worldwide[19]. The results obtained in the present study indicated that class 1 integron was widely disseminated among MDR isolates (74.1%). The frequency of integron in the present study was considerably higher than those reported in United Kingdom (60%)[28], Poland (63.5%)[19], Turkey (6.4%)[15] and Taiwan (71.4%)[29]. It is documented that class 1 integrons are associated with a variety of resistance gene cassettes[19,20]. Given the previous investigations and integron database INTEGRALL (http://integrall.bio.ua.pt/), *aadA* gene cassette confers resistance to streptomycin and spectinomycin; *aadB* gene cassette confers resistance to gentamicin, tobramycin, kanamycin, dibekacin, and sisomicin; bla_{oxa} gene cassette confers resistance to beta-lactam antibiotics; *aacA* gene cassette confers resistance to amikacin, dibekacin, isepamicin, netilmicin, sisomicin and tobramycin; *cmlA6* gene cassette confers resistance to chloramphenicol; *sat2* gene cassette confers resistance to streptothricin; and *VIM, GES, IMP* gene cassettes confer resistance to beta-lactam antibiotics.

In the current study, *aacA4-catB8* and *aadA2-GES-11-IMP-1* were observed to be the most prevalent integron cassettes located on chromosome and plasmid, respectively. Recently, different studies revealed that *aacA4-catB8* is the main cassette of class 1 integron[22,30,31]. In the study conducted by Sung *et al.*[31] in Korea, on 56 MDR *Acinetobacter* spp., it was observed that class 1 integrons were the most prevalent (89.3%). On the basis of the gene cassette nucleotide sequence, Sung *et al.* reported four unique types of gene cassettes from which type A (*aacA4-catB8-aadA1*) was the most prevalent[31]. In 1996–2004 in Taiwan, 283 multidrug resistant *A. baumannii* bloodstream isolates were studied. Class 1 integron was detected in 202 (71.4%) isolates. Neither class 2 nor class 3 integron was detected among these isolates. Among class 1 integron-carrying isolates, seven different types of gene cassettes were identified and *aacA4, catB8*, and *aadA1* were the most prevalent (71.7%) gene cassette types[29]. In another study conducted by Koczura *et al.*[19], in order to investigate the presence of integrons and associated gene cassette in clinical isolates of the *Acinetobacter calcoaceticus-baumannii* complex, sixty-three clinical *Acinetobacter calcoaceticus-baumannii* complex isolates were investigated. The result revealed that none of the isolates harbored class 2 or class 3 integrons. Koczura *et al.* demonstrated that class 1 integrons were detected in 63.5% of isolates and the most common observed gene cassette array was *aacC1-orfA-orfB-aadA1*[19]. The results obtained in the present study indicated that class 2 and class 3 integrons are not the major resistant determinants in multidrug resistant *A. baumannii* isolates, which is in accordance with the previous reports[16,32].

In the current survey, integrons detected contained 1–4 gene cassettes. This finding is consistent with that reported by Gillings *et al.*[33] who stated that class 1 integrons are not typically able to carry more than 6 gene cassettes. As previously mentioned, in the present study, *aacA* gene cassette confers markedly high resistance to aminoglycosides, suggesting a close relationship between high-level resistance rate to aminoglycosides and this gene cassette among the *A. baumannii* isolates.

The most common mechanism of resistance of *A. baumannii* to β-lactam antibiotics is attributed to the presence of a chromosomal cephalosporinase encoding gene[14]. *ampC* (24.7%) gene cassette which confers resistance to cephalosporins was the second most common gene cassette identified in the current study. These findings are consistent with those discussed in the previous studies[24]. Based on literature, carbapenem resistance in *A. baumannii* is most often linked to class D β-lactamases and MBLs. In the current study, the most frequent carbapenem hydrolyzing β-lactamase in class 1 integron-bearing strains were bla_{OXA-23} (9%), bla_{OXA-51} (2.2%), and bla_{OXA-40} (2.2%). The finding is in concordance with those stated in other studies[14,34]. Among *OXA* β-lactamases, *OXA-23* was dominant OXA type and consistent with the reports on other areas in the world[35,36]. The dissemination of MBLs in *A. baumannii* is a threatening incidence. These β-lactamases are able to hydrolyze carbapenems and even every other betalactam antibiotic with the exception of aztreonam[37]. *VIM* and *IMP* are the most frequent MBLs among *A. baumannii* strains. In the present study, gene cassettes encoding *IMP-1* and *VIM-25* were detected among 14 and 9 isolates, respectively. *VIM* and *IMP* have mostly been reported sporadically in some parts of the world[37]. Recently it is documented that GES β-lactamase, as a common Ambler class A β-lactamase in *A. baumannii*, can confer high level resistance to carbapenems. According to results of the present study, *GES-11* and *GES-14* were detected in 15.8% and 11.2% of strains, respectively. These findings are in agreement with those reported in the previous investigations in Turkey and Saudi Arabia[34,38]. *GES-11* prevalence is also reported in Turkey, Egypt, Kuwait, Gaza and France[20,39].

Many reports clarified the presence of *intI* 2 among *A. baumannii* strains. The results obtained in the current survey indicate that 12.5% of the isolates seemed to harbor this class of integrons. Three different cassette arrays were identified in class 2 integrons among *A. baumannii*. In contrast with the previous studies[40], which reported that *IMPs* were usually detected as part of class 1 integron, in the present investigation, *IMP-4* was the most predominant gene cassette in class 1 and 2 integrons which were located on plasmid. In a study conducted in Taiwan, Liu *et al.*[40] investigated 188 *A. baumannii* clinical isolates. They reported that the *blaIMP-1* gene was identified in the gene cassette of class 1 integron in two isolates, which was located on the large plasmids. *VIM-2-VEB-aacA4* was the second most common gene cassette identified in class 2 intergron located on plasmid in the present study. The same gene cassette array was in *A. baumannii* in Poland[41]. *dfrA2-sat2-aadA4* was the third common gene cassette identified in class 2 intergron. The gene cassettes that confer resistance to aminoglycosides among multidrug resistant *A. baumannii* clinical strains have globally been confirmed[19,29]. This finding is consistent with that reported in a study carried out on multidrug resistant *A. baumannii* in Brazil detecting class 2 integrons in 23% of isolates[42], which also carried the main cassette array (*dfrA1-sat2-aadA4*) found in

the present study. Out of 120 multidrug resistant strains, 8 (6.7%) carried class 1 and class 2 integrons simultaneously. To the best of our knowledge, this is the first documentation about existence of gene cassettes in class 1 and 2 integron-bearing *A. baumannii* clinical isolates simultaneously.

To summarize, the present study revealed a high level of *A. baumannii* strains harboring integrons in our hospitals, which may lead to dissemination of multiple antibiotic resistance. The different types of gene cassette arrays in the current study emphasize the key role of geographical features in multidrug resistant isolates distribution which could be attributed to different patterns of antibiotic consumption in distinct areas. Therefore, in order to understand the prevalence and epidemiology of integrons in different molecular types of *A. baumannii*, further studies are required.

Conflict of interest statement

The authors report no conflict of interest.

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