Jundishapur J Microbiol. 2021 June; 14(6):e117766. doi: 10.5812/jjm.117766.

Published online 2021 August 21. **Research Article**

The Occurrence and Characterization of Class I, II, and III Integrons Among Carbapenemase-Producing Clinical Strains of *Acinetobacter baumannii* in Tehran, Iran

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Received 2021 July 10; **Revised** 2021 August 09; **Accepted** 2021 August 09.

Abstract

Background: *Acinetobacter baumannii* has emerged as a critical pathogen with high morbidity and mortality in long-term hospitalized patients who stay in intensive care units. Carbapenemases and integrons are two critical DNA elements that contribute to the emergence of multidrug-resistant (MDR) *A. baumannii*.

Objectives: The current study aimed at characterization and molecular detection of class 1, 2, and 3 integrons among carbapenemase-producing *A. baumannii* strains recovered from a clinical setting in Tehran, Iran.

Methods: A total of 65 non-replicated clinical strains were considered in this study. Class 1, 2, and 3 carbapenemase genes and clonal relatedness of the isolates were investigated by PCR assay.

Results: The prevalence of carbapenemases was as follows: bla_{OXA23} (92.31%), bla_{VIM} (69.23%), and bla_{NDM} (1.54%). In addition, PCR sequencing confirmed the presence of gene cassette arrays consisting of *aacA4-catB8-aadA1* (12/46, 26.09%), *aadB-aadA1* (26.09%, 12/46), *arr2-cmlA5* (30.43%, 14/46), and *dfrA1-aadA1* (7.39%, 8/46) in class 1 integron and *dfrA1-sat2* (52.94%, 9/17), and *sat2-aadA1* (47.06%, 8/17) in class 2 integron. Sequence-based typing of both bla_{OXA-51}-like and *ampC* revealed the following distribution of three different clone types among isolates: clonal complex (CC) 10 (46.15%, 30/65), CC2 (40%, 26/65), and CC3 (13.85%, 9/65). Statistical analysis showed that the presence of the *intI1*, bla_{OXA23}, bla_{VIM}, or bla_{NDM} genes can significantly increase the acquiring MDR phenotypes in *A. baumannii* isolates.

Conclusions: High prevalence of carbapenemase-producing *A. baumannii* harboring integrons is alarming public health. It seems that class 1 integron can be served as a predictive biomarker for the presence of MDR phenotypes in the clinical setting. However, integrons do not carry carbapenemases in these strains.

Keywords: *Acinetobacter baumannii*, Multidrug-Resistant Clinical Isolates, Integrons

1. Background

Antimicrobial resistance (AMR) has become a significant problem with the increasing burden for healthcare systems worldwide. In recent years, antibiotic resistance has been associated with considerable morbidity and mortality rates due to prolonged hospitalization (1). Although the occurrence of antibiotic resistance is a natural phenomenon in bacteria, it is exacerbated by the misuse of antibiotics in humans and animals (2). A significant reason for the rapid spread of antibiotic resistance is the highly mobile genetic elements. These elements can replicate and pass among bacterial species (3). *Acinetobacter baumannii* is a common nosocomial pathogen that causes different infections, such as ventilator-associated pneumonia, bacteremia, urinary tract infections, surgical site infections, and secondary meningitis in hospitalized patients, especially those with immunodeficiency (4). The relatively recent emergence and increased prevalence of multidrug-resistant (MDR) *A. baumannii* has been an issue of great concern. The World Health Organization (WHO) lists *A. baumannii* as a critical pathogen, highlighting the need to develop new and effective antibiotics (5).

Miserably, the number of MDR *A. baumannii* isolates

has increased significantly worldwide. Also, *A. baumannii* can acquire or upregulate resistance genes through genomic plasticity, limiting effective therapeutic options and increasing mortality rates. This phenomenon can increase resistance to multiple antibiotics, including those used as a last resort, such as carbapenems, reserved for cases where all alternatives have been depleted (6). The enzymes IMP, VIM, GIM, SIM, and NDM are classified as class (B) Metallo-β-lactamases (MBLs), and their genes are mostly found in transmissible plasmids (7).

In *Acinetobacter* spp., the acquisition and spread of an antimicrobial-resistant determinant in hospitals and communities are often facilitated by horizontal gene transfer of mobile genetic elements, including plasmids, transposons, and integrons. Among these mobile elements, integrons are unique for their ability to carry and express resistance genes (8). It has been suggested that multidrug-resistant strains acquire their antibiotic-resistant genes via integrons that take single or multiple gene cassettes (9). Integrons carrying different cassette arrays have been reported in several studies from South America to Far East Asia (10).

2. Objectives

The current study aimed at characterizing class 1, 2, and 3 integrons among clinical carbapenemase-producing *A. baumannii* isolates from hospitalized patients in Tehran, Iran, followed by the genotypic analysis of these isolates.

3. Methods

3.1. Collection and Identification of Bacterial Isolates

In this cross-sectional study, a total of 103 consecutive non-duplicate *A. baumannii* isolates collected from clinical specimens in hospitalized patients from an educational hospital in Tehran, Iran, from November 2019 to July 2020 were investigated. The isolates were obtained from sputum, tracheal aspirate, wound, catheter, and cerebrospinal fluid (CSF). Standard microbiological and biochemical tests, including triple sugar iron agar (TSI), indole, methyl red (MR), Voges–Proskauer (VP), citrate (IMVIC), and oxidase test were used to identify *A. baumannii* isolates. All K/K colonies on TSI and oxidase negative coccobacilli (11) were genotypically confirmed as *A. baumannii* by the presence of the $bla_{\text{OXA-51}}$-like (1) and rpoB PCR sequencing (12).

3.2. Antibiotic Susceptibility Testing

According to the Clinical and Laboratory Standards Institute (CLSI), the antibiotic susceptibility profiles of *A. baumannii* isolates were determined using the disk diffusion method. In this step, the results were interpreted with criteria published in CLSI 2019 (13). For this purpose, various antibiotic disks, such as ampicillin/sulbactam (SAM, 10 μg), minocycline (MN, 30 μg), meropenem (MEM, 10 μg), amikacin (AN, 30 μg), ciprofloxacin (CIP, 5 μg), trimethoprim-sulfamethoxazole (SXT, 1.25 + 23.75 μg), and ceftazidime (CAZ, 30 μg) were used. In the next step, minimum inhibitory concentrations (MICs) were determined for imipenem in carbapenem-resistant isolates using E-test (bioMérieux). Antibiotic susceptibility was interpreted based on CLSI clinical breakpoints. The *Escherichia coli* ATCC 25922 was used as a quality control strain. The categorizations of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) *A. baumannii* were performed based on Magiorakos criteria (14).

3.3. Detection of Genes Encoding β-lactamase, Integrases, and Clonal Complex Analysis

DNAs of bacterial isolates were extracted as previously described (15). A PCR experiment was used to determine the presence of genes producing carbapenemases and integrases using primers targeting $bla_{\text{OXA-23}}$-like, bla_{VIM}, bla_{NDM}, *intI1*, *intI2*, and *intI3* genes (Table 1). The PCR conditions were based on the mentioned reference (16). All isolates were confirmed as *A. baumannii* by sequencing of the $bla_{\text{OXA-51}}$-like, an intrinsic enzyme marker, and *ropB*, as described previously (15). Determination of the allele number and detection of the clonal complex (CC) for each isolate were performed by a combination of sequence-based typing (SBT) of $bla_{\text{OXA-51}}$-like and *ampC* as reported previously (15).

3.4. PCR Amplification and Sequencing of Integrons Internal Variable Region

All integron–positive MDR *A. baumannii* strains were assessed for variable regions of Class 1 - 2 integrons by the primers 5′-CS/3′-CS. The PCR conditions were based on the mentioned reference (9). Sequencing of the purified PCR amplicons was performed using DNA analyzers (Applied Biosystems, Inc.). The nucleotide sequence analysis was performed by the BLAST tool at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (22). The sequences were manually analyzed using CLC main workbench software version 20 (CLC Bio, Aarhus, Denmark).

Table 1. Primer Pairs Used for PCR Amplification and Sequencing

Target	Primer Sequence (5´-3´)	Product Size (bp)	Reference
rpoB		1024	(12)
F	CTGACTTGACGCGTGA		
R	TGTTTGAACCCATGAGC		
***bla*$_{OXA-51}$-like**		501	(17)
F	GATCGGATTGGAGAACCAGA		
R	ATTTCTGACCGCATTTCCAT		
***bla*$_{NDM}$**		155	(18)
F	GCGCAACACAGCCTGACTTT		
R	CAGCCACCAAAAGCGATGTC		
***bla*$_{VIM}$**		518	(19)
F	GGGAGCCGAGTGGTGAGT		
R	GGCACAACCACCGTATAG		
intI1		250	(20)
F	TCTCGGGTAACATCAAGG		
R	AGGAGATCCGAAGACCTC		
intI2		789	(21)
F	CACGGATATGCGACAAAAAGGT		
R	GTAGCAAACGAGTGACGAAATG		
intI3		980	(21)
F	GCCTCCGGCAGCGACTTTCAG		
R	ACGGATCTGCCAAACCTGACT		
Conserved segment of class 1 integrons		Variable	(21)
5′-CS	GGCATCCAAGCAGCAAG		
3′-CS	AAAGCAGACTTGACCTGA or GAAGCGGCGTCGGCTTGA		
Conserved segment of class 2 integrons		Variable	(21)
5′-CS	ACCTTTTTGTCGCATATCCGTG		
3′-CS	TACCTGTTCTGCCCGTATCT		

3.5. Statistical Analyses

The normality of continuous data distribution was assessed by the Kolmogorov-Smirnov test. Numerical data were summarized as means and standard deviations or median and interquartile range as appropriate. Categorical data were summarized as frequencies and proportions. The association of variables was analyzed using one-way analysis of variance, student t-test, and Mann-Whitney U Kruskal-Wallis tests as appropriate. All statistical analyses were conducted with STATA 12.0 (StataCorp LP, College Station, TX, USA), SPSS for Windows version 24.0 (IBM Corp., Armonk, NY), and GraphPad Prism software version 8.0 (GraphPad Software Inc., La Jolla, CA, USA). A $P < 0.05$ was defined as statistical significance in all tests.

4. Results

4.1. Collection and Identification of Bacterial Isolates

A total of 65 non-repetitive isolates were collected and confirmed as MDR *A. baumannii* by phenotypic and genotypic antimicrobial methods. The isolates were recovered from clinical specimens, including tracheal aspirate (n = 57/65; 87.69%), sputum (n = 3/65; 4.62%), catheter (n = 3/65; 4.62%), CSF (n = 1/65; 1.54%), and wound (n = 1/65; 1.54%). The mean age of the patients was 48.25 ± 21.09 years (ranging from 5 to 96). Also, 41 patients (63.1%) were male, and 24 cases (36.9%) were female (Table 2). Antimicrobial susceptibility results for the 65 *A. baumannii* isolates are shown in Table 2. The isolates showed MDR phenotypes and resistance to most of the tested antibiotics by the disk dif-

fusion method, in particular high-level resistance to AN (n = 64; 98.46%), SXT (n = 63; 96.92%), CIP (n = 62; 95.38%), MEM (n = 60; 92.31%), and CAZ (n = 57; 87.69%) (Figure 1). The imipenem MIC_{90} for all isolates was ≥16 mg/L. Quite alarmingly, 5 isolates (7.69%) were resistant to all antibiotics tested. Surprisingly, 55 (84.62%) and 35 (53.85%) out of 65 isolates were susceptible to MN and SAM, respectively. Notably, the frequency of MDR isolates was higher in non-ICU wards rather than ICU (Figure 2).

4.2. Detection of β-lactamases Encoding Genes

The detected carbapenemase (bla_{OXA23}) and Metallo-β-lactamases (bla_{VIM} and bla_{NDM}) are listed in Table 2. The bla_{OXA23} was detected in 92.31% (60/65) isolates using PCR amplification. The bla_{VIM} and bla_{NDM} were detected in 69.23% (45/65) and 1.54% (1/65) of the isolates, respectively. Data analysis showed that the presence of bla_{OXA23} in strains caused a 4- (95% CI, 1.07 - 14.57) and 14-fold (95% CI, 4.22 - 47.50) increase (significantly) in the odds of resistance to SAM and MEM and also the presence of bla_{VIM} caused a 3.5- (95% CI, 1.48 - 8.59) and 11-fold (95% CI, 3.90 - 33.67) increase (significantly) in the odds of resistance to SAM and MEM, respectively.

4.3. PCR Amplification and Characterization of Class 1-3 Integrons

The presence of integrase genes, *intI1*, *intI2*, and *intI3* were detected by PCR in 70.77% (46/65), 26.15% (17/65), and 0% (0/65) of MDR *A. baumannii* isolates, respectively. The class 1 and 2 integrons were widespread among clinical isolates. The *intI3* was not detected in any of the strains. Cassette arrangements of class 1 and 2 integrons were characterized by PCR sequencing of gene cassettes in the internal variable regions of integrons. Sequencing confirmed the presence of cassette arrays consisting of *aacA4-catB8-aadA1* (12/46, 26.09%), *aadB-aadA1* (12/46, 26.09%), *arr2-cm1A5* (14/46, 30.43%), and *dfrA1-aadA1* (8/46, 7.39%) in class 1 integron and *dfrA1-sat2* (9/17, 52.94%) and *sat2-aadA1* (8/17, 47.06%) in class 2 integron (Figure 3).

There was no significant difference in resistance to the studied antibiotics among *intI1*-positive and *intI1*-negative isolates. The *intI1* negative isolates displayed 26.7%, 29.7%, 29%, 30.2%, 24.6%, and 40% resistance rate to SAM, AN, CIP, SXT, CAZ, and MN, respectively, compared with *intI1*-positive isolates. On the other hand, there was a statistically significant relationship ($P < 0.05$) between resistance to the tested antibiotics and the lack of the *intI2* gene. The *intI2*-negative isolates displayed 73.3%, 73.4%, 74.2%, 73%, 71.9%, and 50% resistance rate to SAM, AN, CIP, SXT, CAZ, and MN, respectively, compared with *intI2* positive isolates. Also, the rate of MDR phenotype in *A. baumannii* isolates positive for the *intI1*, bla_{OXA23}, bla_{VIM}, and bla_{NDM} was statistically significant. In addition, although the probability of acquired MDR phenotype for the *intI2*-positive isolates was 2.7-fold (95% CI, 0.8-8.6) higher than *intI2*-negative isolates, the latter value was not statistically significant ($P > 0.05$) (Figure 4).

4.4. Sequence-Based Typing of blaOXA-51-like and ampC Alleles

Sequence-based typing of both bla_{OXA-51}-like and *ampC* is a discriminatory and reliable method that can distinguish *Acinetobacter* isolates at the level of clonal complex (23). The SBT results revealed the following distribution of three different clone types among MDR isolates, including CC10 (46.15%, 30/65), CC2 (40%, 26/65), and CC3 (13.85%, 9/65), as shown in Table 2. Overall, 25 out of 30 isolates (83.33%) in CC10, 22 out of 26 isolates (84.62%) in CC2, and 8 out of 9 isolates (88.89%) in CC3 showed MIC ≥16 mg/L for imipenem. Therefore, CC2 and CC10 showed a high level of imipenem resistance. Data analysis showed a heterogenic structure in integron cassette arrays within CCs. The distribution of cassette arrays in class 1 and 2 integrons within clonal complexes is shown in Table 2.

4.5. Nucleotide Accession Numbers

DNA sequences of gene cassette arrays consisting of *aacA4-catB8-aadA1* (GenBank accession number = MZ508285), *aadB-aadA1* (MZ508283), *arr2-cm1A5* (MZ508286), and dfrA1-aadA1 (MZ508284), in class 1 integron and *dfrA1-sat2* (MZ508287) and *sat2-aadA1* (MZ508288) in class 2 integron were deposited in GenBank database.

5. Discussion

Infections associated with MDR bacterial strains have become one of the leading causes of morbidity and mortality worldwide (24). Integrons as transposon-like genetic elements are conserved and encode antibiotic resistance determinants and have a high capacity for chromosomal integration in bacteria (25, 26). To date, several classes of integrons have been described, of which class 1 and 2 integrons are commonly reported from MDR *A. baumannii* strains (27). Carbapenems are usually the antibiotic of choice against *A. baumannii* strains. However, the rate of resistance to carbapenems in this bacterium is increasing day by day. Resistance to carbapenems can be due to various mechanisms, such as producing the enzymes, including Metallo-β-lactamase and oxacillinase (28, 29).

According to our results, most MDR *A. baumannii* isolates were obtained from the tracheal aspirate samples. Consistent with our research, in a study conducted by Souza et al., *A. baumannii* was the most frequently isolated

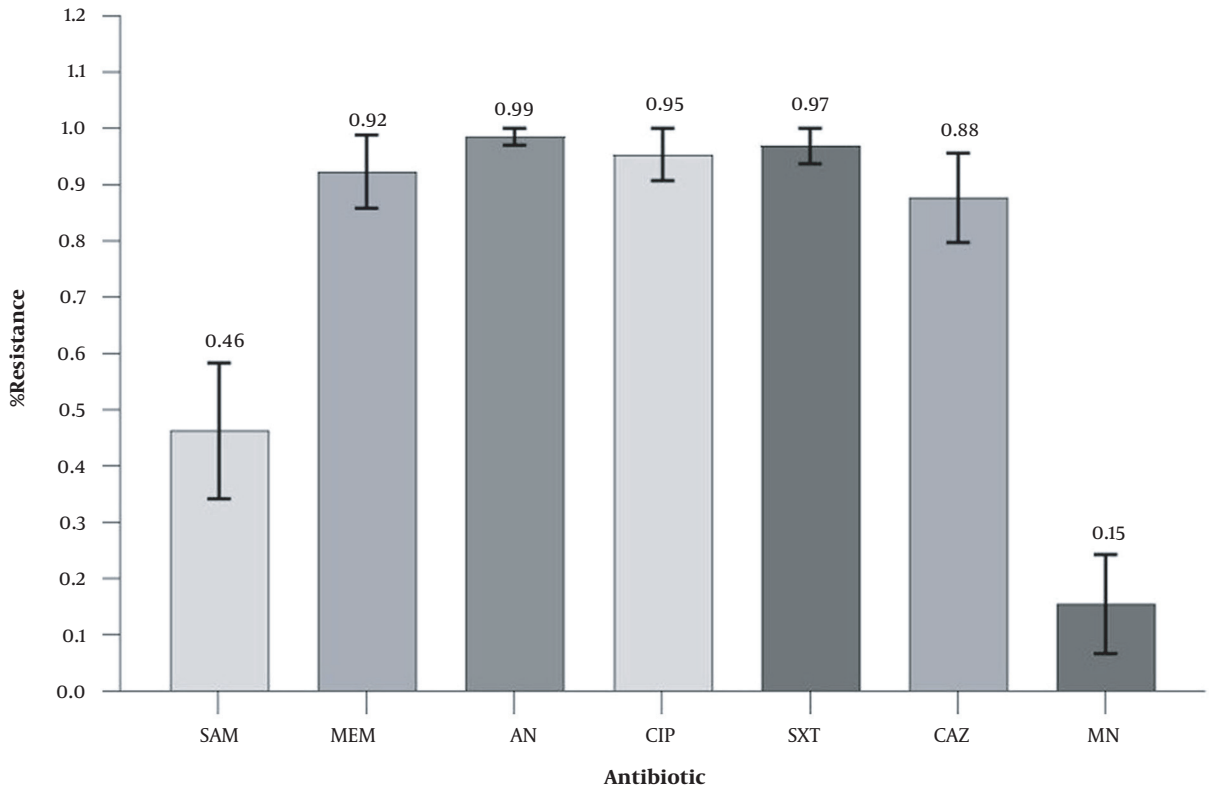

Figure 1. Antibiotics resistance patterns in *Acinetobacter baumannii* isolates. Notably, out of 65 isolates, 10 (15.38%) and 30 (46.15%) strains were susceptible to MN and SAM, respectively. Bars represent mean ± standard deviation (SD). The numbers on the bars represent percentages.

bacterial species in the tracheal secretion of patients with ventilator-associated pneumonia (30). However, Barbier et al. reported that the most frequent pathogens associated with ventilator-associated pneumonia were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (31). The ICU has been described as the main center of antibiotic resistance development, with increasingly resistant isolates complicating the treatment of MDR infections in ICU patients (32). Surprisingly, the frequency of MDR *A. baumannii* isolates was higher in non-ICU wards rather than ICU. This is alarming and indicates that MDR isolates have been circulated in our hospital, and urgent attention and application of preventive protocols are needed to reduce such a fearful threat in hospitalized patients. In this study, the highest antibiotic resistance was related to amikacin, trimethoprim + sulfamethoxazole, and ciprofloxacin, respectively. Also, *A. baumannii* strains producing aminoglycoside modifying enzymes (AMEs) are highly resistant to different aminoglycosides, such as gentamicin, amikacin, and tobramycin. Similar to our findings, Cho et al. reported aminoglycoside resistance genes in 81% *Acinetobacter* isolates from two Korean hospitals (33).

In this study, the imipenem MIC_{90} for all isolates was $\geq$ 16 mg/L and showed resistance to carbapenems. According to a study conducted by Lee et al., isolates with MIC $\leq$ 4 mg/L were susceptible to carbapenem, and those with MIC $\geq$ 8 mg/L were resistant in patients with *A. baumannii* bacteremia (34). Consistent with our results, Akbari Dehbalaei et al. reported that resistant to carbapenems was up to 85% in *A. baumannii* isolates (35). Unfortunately, in this study, 7.69% of the isolates were resistance to all tested antibiotics, which will be a significant obstacle to effective treatment in the future. Therefore, antibiotic usage should be controlled to prevent this serious threat. On the other hand, 84.62% and 53.85% of isolates were susceptible to MN and SAM, respectively. This result indicated that these two antibiotics could be effective for the treatment of *A. baumannii* infections in combination form. However, excessive usage of these two antibiotics can also increase antibiotic resistance against them.

In this study, $bla_{\text{OXA-23}}$, bla_{VIM}, and bla_{NDM} were detected

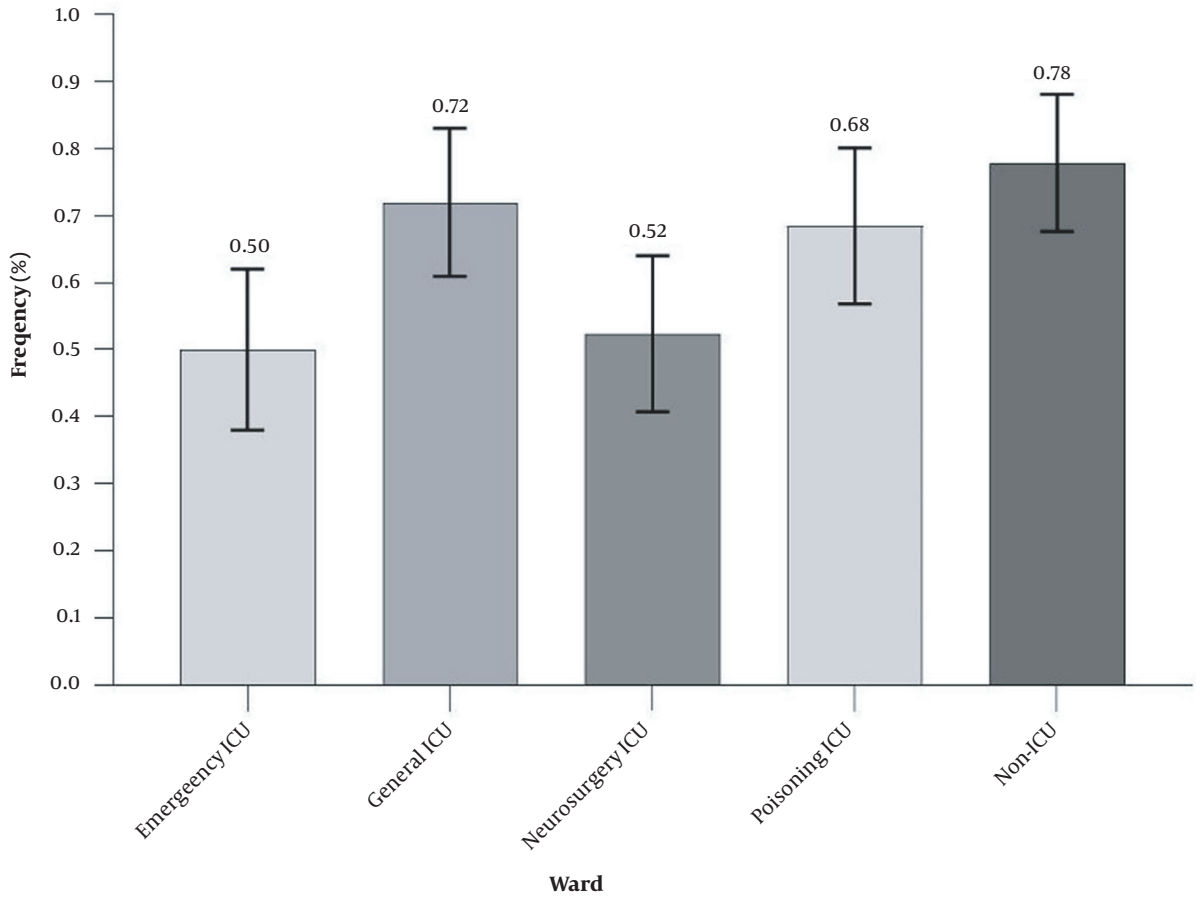

Figure 2. The prevalence of *Acinetobacter baumannii* isolates obtained from different ICU and non-ICU wards. General ICU had the highest share of clinical samples (72%), while the emergency ICU had the least share of clinical samples (50%). Bars represent mean ± standard deviation (SD). The numbers on the bars represent percentages. ICU, intensive care unit.

with high frequency in *A. baumannii* isolates. The $bla_{\text{OXA-23}}$-like gene is one of the most prevalent β-lactamase genes in the carbapenem-resistant *A. baumannii* genome, mostly on plasmids (36). Specific and quick identification of *A. baumannii* and strains containing the $bla_{\text{OXA-23}}$-like gene will reference information on treatment and control measures for carbapenem resistance (37). Ning et al. showed that ST191 and ST195 isolates of OXA-23-producing *A. baumannii* could spread in a hospital and became potential nosocomial outbreak strains. In this regard, they suggested that antimicrobial management and surveillance of imipenem-resistant *A. baumannii* should be improved (38). Moreover, in the study by Akbari Dehbalaei et al., the $bla_{\text{OXA-23}}$ gene was detected in 81.81% of the isolates.

This study concluded that highly resistant $bla_{\text{OXA-23}}$ gene-harboring endemic clones of *A. baumannii* were disseminated in the ICUs of two studied hospitals (35). The bla_{VIM} is another β-lactamases encoding genes with a frequency of 69.23% in this study. The frequency of this gene was reported to be 17.44% and 18.18% in other studies conducted in Iran in 2014 and 2016, respectively (39, 40). Comparison of these results showed that the frequency of this gene had increased significantly in recent years in Iran (39, 40). Therefore, it seems necessary to find new treatments to deal with this problem. In this study, only one isolate harbored the $bla_{\text{NDM-1}}$ gene. Pillonetto et al. presented the first instance of *A. baumannii* sequence type 25 generating $bla_{\text{NDM-1}}$, isolated from the urinary tract of a 71-year-old man in Brazil (41). Bonnin et al. recently suggested that *A. baumannii* may accept resistant genes and act as a gene donor passing resistance genes to other bacteria, including *Enterobacteriaceae* (42). It seems that the MDR phenotype in *A. baumannii* is associated with the cooperation of carbapenemases, class 1 integrons, and possibly efflux pumps.

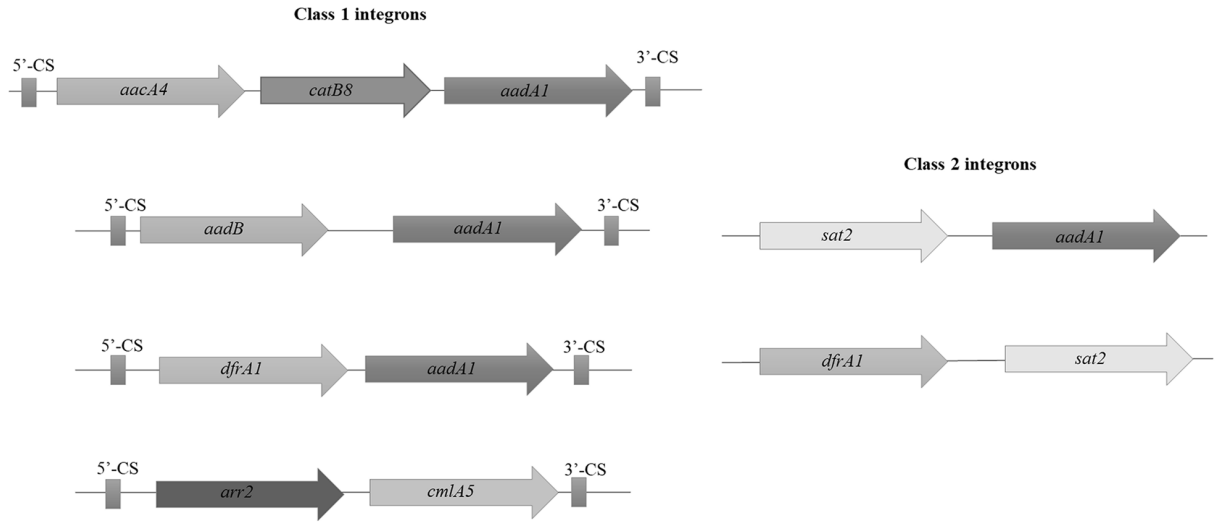

Figure 3. The genetic maps of class 1 and 2 integrons in clinical isolates. PCR sequencing confirmed the presence of gene cassette arrays consisting of *aacA4-catB8-aadA1* (12/46, 26.09%), *aadB-aadA1* (12/46, 26.09%), *arr2-cm1A5* (14/46, 30.43%), and *dfrA1-aadA1* (8/46, 7.39%) in class 1 integron (left hand) and *dfrA1-sat2* (9/17, 52.94%) and *sat2-aadA1* (8/17, 47.06%) in class 2 integron (right hand).

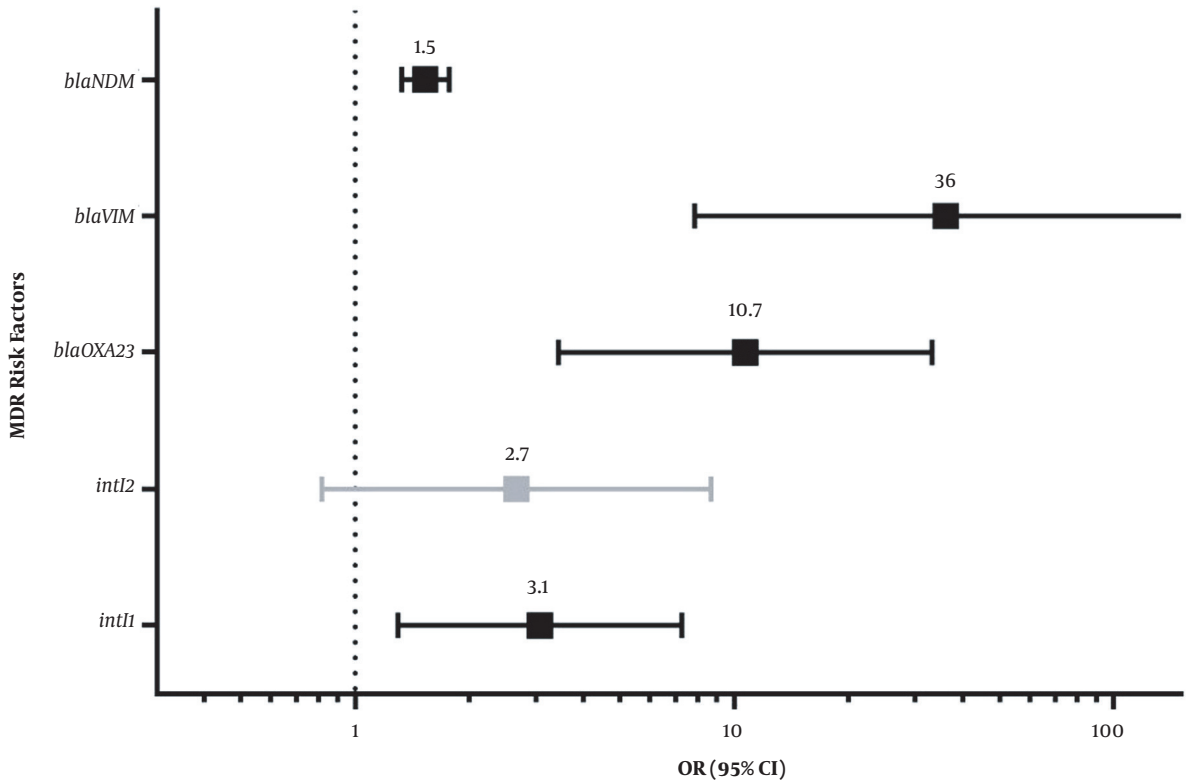

Figure 4. Odds ratio and 95% confidence interval of risk factors (having the *intI1*, bla_{OXA23}, bla_{VIM}, bla_{NDM}, or *intI2*) for acquiring multidrug-resistant phenotypes in *Acinetobacter baumannii* isolates. The numbers on the bars show the probability of acquired MDR phenotype in each considered risk factor. All of these risk factors were statistically significant except *intI2*. The dotted vertical line shows a significance threshold.

The presence of integrase genes, *intI1* and *intI2*, was detected by PCR in 70.77% and 26.15% of *A. baumannii* isolates, respectively. These data indicated that class 1 and 2 integrons were widely distributed among clinical isolates of *A. baumannii*. The *intI3* was not detected in any of the strains. Similar to our study, Goudarzi and Azimi reported class 1 and 2 integrons in 66.7% and 20% of isolates, respectively. However, the class 3 integron was detected in three *A. baumannii* strains (8). Moreover, Nourbakhsh et al. reported the frequency of class 1, 2, and 3 integrons to be 100%, 44%, and 3%, respectively, among *A. baumannii* isolates (43). The sequence-based typing results of $bla_{\text{OXA-51}}$-like and *ampC* alleles revealed the following distribution of three different clone types among MDR isolates, including CC10 (46.15%), CC2 (40%), and CC3 (13.85%). In the study by Nazari et al., a comparison of clonal relatedness between clinical and non-clinical isolates illustrated that widespread clones, including CC2, CC3, and CC10 were common clonal complexes among clinical and non-clinical strains (15). In addition, a systematic review on clonal relatedness of *A. baumannii* isolated from the Middle East showed that CC2 was the most prevalent clonal complex isolated from Lebanon, Palestine, Saudi Arabia, Turkey, Yemen, Iran, Iraq, and Kuwait. In this study, CC2 and CC10 showed a high-level imipenem resistance (44).

5.1. Conclusions

The high prevalence of carbapenemase-producing *A. baumannii* isolates in the ICU requires a rigorous antimicrobial stewardship and infection control program. Class 1 and 2 integrons in clinical strains are repertoires of aminoglycoside-modifying enzymes. Class 1 integron can be served as a predictive biomarker for the presence of MDR bacteria in the clinical setting. However, hoarding of carbapenemases on the integron apparatus is not widespread among *A. baumannii* strains. Continuous surveillance MDR *A. baumannii* and elucidation of their AMR mechanisms in the clinical setting are clearly necessary to help develop effective therapy regimens and to prevent the further dissemination of these superbug bacteria. Further studies are required to elaborate the association of gene pools in *A. baumannii* and antibiotic resistance patterns with epidemic and clinical outcomes of infection.

Footnotes

Authors' Contribution: Omid Azizi, Sepideh Fereshteh, and Seyed Mahmmoud Barzi collected the samples and their data; Sepideh Fereshteh, Seyed Mahmmoud Barzi, and Omid Nasiri carried out other phenotypic and genotypic tests; Mohammad Ghorbani: Analyzed the data; Omid Azizi, Sepideh Fereshteh, and Farzad Badmasti wrote the manuscript; Farzad Badmasti, supervised the project and wrote and revised the manuscript.

Conflict of Interests: The authors declared that they have no conflicts of interest.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: This project was done based on ethical rules as already endorsed by the Pasteur Institute of Iran (Ethic No.: IR.PII.REC.1397.015). The consent form was not obtained from patients since the clinical isolates were collected from the clinic lab, and the data were obtained in a blind way.

Funding/Support: No funding was allocated for this study.

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Table 2. Demographic Data on Samples and 65 Non-duplicated Multidrug-Resistant *Acinetobacter baumannii* Isolates Recovered from Hospitalized Patients

Isolate No.	Age/Sex	Ward	Outcome	Isolation source	Resistance Patterns	MIC_{IMP}	bla_{OXA23}	bla_{VIM}	bla_{NDM}	*intI1*	Class 1 Integron Cassette Arrays	*intI2*	Class 2 Integron Cassette Arrays	*IntI3*	Clonal Complex
1	42/M	Poisoning ward	ND	S	SAM-MEM-AN-CIP-SXT-CAZ	8	+	-	-	+	*aadB-aadA1*	-	-	-	CC2
2	63/M	Neurosurgery ICU	Death	T	MEM-AN-CIP-SXT-CAZ	> 64	-	+	-	+	*aadB-aadA1*	-	-	-	CC10
3	50/M	Emergency ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*dfrA1-aadA1*	-	-	-	CC2
4	50/F	Emergency ICU	Death	T	MN-SAM-MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*dfrA1-aadA1*	+	*sat2-aadA1*	-	CC2
5	40/M	Poisoning ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*arr2-cmlA5*	+	*dfrA1-sat2*	-	CC3
6	41/M	Poisoning ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*dfrA1-aadA1*	-	-	-	CC10
7	75/M	General ICU	Death	T	SAM-AN-CIP-SXT-CAZ	8	+	+	-	+	*arr2-cmlA5*	-	-	-	CC10
8	50/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC10
9	42/M	General ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	8	-	-	-	+	*dfrA1-aadA1*	+	*sat2-aadA1*	-	CC3
10	25/F	General ICU	Death	T	MEM-AN-CIP-SXT-CAZ	32	+	+	-	-	-	-	-	-	CC10
11	25/F	General ICU	Death	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*dfrA1-aadA1*	-	-	-	CC3
12	37/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	-	-	+	*dfrA1-sat2*	-	CC3
13	22/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*arr2-cmlA5*	-	-	-	CC10
14	37/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*dfrA1-aadA1*	-	-	-	CC10
15	29/M	Neurosurgery ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	32	+	-	-	+	*arr2-cmlA5*	+	*dfrA1-sat2*	-	CC3
16	18/F	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*arr2-cmlA5*	-	-	-	CC10
17	25/F	General ICU	Death	C	MN-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*arr2-cmlA5*	-	-	-	CC2
18	49/M	Neurosurgery ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*arr2-cmlA5*	+	*dfrA1-sat2*	-	CC2
19	40/F	Neurosurgery ward	Discharge	C	SAM-MEM-AN-CIP-SXT-CAZ	16	+	-	-	-	-	+	*sat2-aadA1*	-	CC2
20	96/M	Infectious ward	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*dfrA1-aadA1*	-	-	-	CC2
21	32/F	General ICU	Death	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*arr2-cmlA5*	+	*dfrA1-sat2*	-	CC10
22	80/M	Neurosurgery ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*dfrA1-aadA1*	-	-	-	CC10

23	5/M	General ICU	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC10
24	60/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*arr2-cmlA5*	-	-	-	CC2
25	32/F	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*arr2-cmlA5*	-	-	-	CC10
26	65/F	Emergency ICU	Death	T	MEM-AN-CIP-SXT-CAZ	> 64	+	-	-	-	-	-	-	-	CC2
27	75/M	General ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*arr2-cmlA5*	+	*sat2-aadA1*	-	CC10
28	94/M	Infectious ward	Death	T	MN-SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*aacA4-catB8-aadA1*	+	*sat2-aadA1*	-	CC2
29	58/M	Poisoning ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC10
30	33/F	General ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*aadB-aadA1*	-	-	-	CC2
31	29/M	Neurosurgery ICU	Discharge	T	MEM-AN-CIP-SXT	8	+	-	-	-	-	-	-	-	CC10
32	80/M	Neurosurgery ICU	Discharge	S	MEM-AN-CIP-SXT-CAZ	>64	+	+	-	-	-	-	-	-	CC10
33	16/F	Poisoning ICU	Discharge	T	MN-MEM-AN-CIP-SXT-CAZ	16	+	-	-	-	-	-	-	-	CC2
34	74/F	General ICU	Death	T	SAM-MEM-AN-CIP-SXT	8	+	-	-	+	*aacA4-catB8-aadA1*	-	-	-	CC10
35	25/M	Poisoning ICU	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	-	-	-	-	-	CC3
36	58/M	Poisoning ICU	Death	T	SAM-AN-SXT	> 64	+	+	-	+	*aadB-aadA1*	+	*sat2-aadA1*	-	CC10
37	55/M	Poisoning ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	-	-	-	-	-	CC10
38	74/F	General ICU	Death	T	MEM-AN-CIP-SXT-CAZ	8	+	-	-	+	*aadB-aadA1*	-	-	-	CC10
39	33/F	General ICU	Death	T	MEM-AN-CIP-SXT-CAZ	32	+	+	-	-	-	-	-	-	CC2
40	40/M	Neurosurgery ICU	Discharge	T	MN-MEM-AN-CIP-SXT	8	+	-	-	-	-	-	-	-	CC2
41	9/F	General ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	+	-	-	-	-	-	-	CC10
42	62/F	General ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	-	-	-	-	-	CC10
43	44/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*aadB-aadA1*	+	*sat2-aadA1*	-	CC10
44	40/F	Poisoning ICU	Death	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	-	-	-	-	-	CC2
45	69/M	General ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	32	+	-	-	+	*aadB-aadA1*	+	*sat2-aadA1*	-	CC2
46	40/F	Poisoning ICU	Death	T	MEM-AN-CIP-SXT	8	+	+	-	-	-	-	-	-	CC2
47	69/F	General ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC2

48	80/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	-	-	-	-	-	CC10
49	41/M	Poisoning ICU	Discharge	T	MN-SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	-	-	-	-	-	CC2
50	92/M	General ICU	Death	T	MN-MEM-AN-CIP-SXT-CAZ	32	+	+	-	-	-	+	*dfrA1-sat2*	-	CC2
51	45/M	Neurosurgery ICU	Discharge	W	MEM-AN-CIP-CAZ	8	+	-	-	+	*arr2-cmlA5*	-	-	-	CC2
52	50/F	Poisoning ICU	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	-	-	-	-	-	CC10
53	55/M	Poisoning ICU	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC2
54	45/M	Neurosurgery ICU	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*arr2-cmlA5*	-	-	-	CC2
55	69/M	Poisoning ICU	Death	S	MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*arr2-cmlA5*	+	*dfrA1-sat2*	-	CC10
56	68/F	Neurology ward	Discharge	C	AN-CIP-SXT-CAZ	32	-	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC3
57	19/M	Neurosurgery ward	Discharge	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC3
58	65/M	Poisoning ICU	Discharge	T	MEM-AN-CIP-CAZ	8	-	-	+	+	*aadB-aadA1*	-	-	-	CC10
59	58/M	Neurosurgery ward	Discharge	CF	SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*aadB-aadA1*	-	-	-	CC10
60	46/F	Poisoning ICU	Death	T	MN-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*aacA4-catB8-aadA1*	+	*dfrA1-sat2*	-	CC2
61	55/M	Poisoning ICU	Death	T	MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*aadB-aadA1*	-	-	-	CC10
62	58/F	Neurosurgery ICU	ND	T	SAM-MEM-AN-CIP-SXT-CAZ	16	+	-	-	+	*aadB-aadA1*	-	-	-	CC10
63	17/M	Poisoning ICU	Discharge	T	MN-SAM-MEM-AN-CIP-SXT-CAZ	32	+	+	-	+	*aacA4-catB8-aadA1*	+	*dfrA1-sat2*	-	CC2
64	35/M	Neurosurgery ICU	Discharge	T	CIP-SXT-CAZ	32	-	+	-	+	*aadB-aadA1*	-	-	-	CC3
65	31/F	General ICU	Death	T	MN-SAM-MEM-AN-CIP-SXT-CAZ	> 64	+	+	-	+	*aacA4-catB8-aadA1*	-	-	-	CC2

Abbreviations: SAM, ampicillin/sulbactam; MN, minocycline; MEM, meropenem; AN, amikacin; CIP, ciprofloxacin; SXT, trimethoprim- sulfamethoxazole; CZ, ceftazidime; ND, not determined; S, sputum; T, tracheal; CF, CSF; C, catheter; W, wound.