Evaluating the frequency of carbapenem and aminoglycoside resistance genes among clinical isolates of Acinetobacter baumannii from Ahvaz, south-west Iran

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Abstract

Acinetobacter baumannii is one of the most important opportunistic challenging pathogens as a result of its ability to acquire resistance to broad range of antibiotics and cause a variety of severe nosocomial infections. We investigated the frequency of the aminoglycoside-modifying enzymes (AMEs) and oxacillinase genes among clinical isolates of A. baumannii collected from hospitalized patients in Imam Khomeini Hospital, Ahvaz city, Iran. This prospective cross-sectional study was performed on 80 clinical isolates of A. baumannii collected from patients referred to Imam Khomeini Hospital in Ahvaz, Iran. Initial identification of isolates as A. baumannii was performed using conventional bacteriologic tests, and final confirmation was carried out by PCR of blaOXA-51-like gene and multiplex PCR of gyrB locus. MICs of different classes of antibiotics against these strains was measured by using VITEK 2 system. After extraction of genomic DNA, two groups of multidrug-resistant A. baumannii genes including AME (aadA1, aadB, aphA6 and aacC1) and oxacillinases (blaOXA-23-like, blaOXA-51-like, blaOXA-24-like, blaOXA-25-like and blaOXA-143-like) were detected. According to antibiotic susceptibility testing, among 80 A. baumannii strains, 75 isolates (91.25%) were multidrug resistant. The results showed that colistin and tigecycline, with respective sensitivity rates of 97.5% (78/80) and 56.25% (45/80), had the highest effects. The presence of blaOXA-51-like and gyrB genes was confirmed in all strains. Furthermore, blaOXA-23-like and blaOXA-24-like genes were found in 68.75% (55/80) and 20% (16/80) of isolates respectively, while no isolate harbored the blaOXA-143-like gene. The frequency of genes encoding the AMEs including aadA1, aacC1, aphA6 and aadB were 11.25% (9/80), 16.25% (13/80), 22.5% (18/80) and 30% (24/80) respectively. Our findings indicate that the presence of aadB and aphA6 is correlated with high resistance against amikacin and gentamicin. We found a very high resistance rate against most of the antimicrobial agents usually prescribed for severe infections caused by A. baumannii. Therefore, because of rapid emergence of resistance even for colistin or tigecycline, monotherapy should be avoided. These results show the importance of providing antibiotics correctly in intensive care units and following antibiotic stewardship protocols as the only effective strategies to attempt to control antibiotic resistance in healthcare settings.

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Keywords: Acinetobacter baumannii, aminoglycoside resistance genes, carbapenem-resistant A. baumannii (CRAB), multidrug resistance (MDR), oxacillinase genes

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Introduction

Acinetobacter baumannii is an opportunistic pathogen that can survive for long periods on both dry and moist surfaces. A. baumannii is prevalent in healthcare facilities, colonizes different surfaces and survives on the hair or skin of patients.
and hospital staff as a commensal bacterium. The ability of A. baumannii to survive in hospital environments for a long time and its ability to gain many virulence factors have led it to emerge as an important nosocomial pathogen [1,2].

Multidrug resistance (MDR) is defined as resistance to three or more representatives of the following classes of antibiotics: carbapenems, aminoglycosides, third-generation cephalosporins and fluoroquinolones. The rapid spread of MDR isolates of A. baumannii causing nosocomial infections is of great global concern [3]. Carbapenems are members of the ß-lactam class of antibiotics, which are used as a drug of choice for treatment of infections due to MDR A. baumannii isolates, but recently the rise of resistance to this class of antibiotics has led to limited efficiency of these drugs [4].

Carbapenem-resistant A. baumannii is a major cause of nosocomial infections worldwide, leading to high mortality and morbidity and consequently increased medical costs. Resistance to carbapenem in A. baumannii strains is mediated by acquisition of a class B or class D ß-lactamase genes such as metallo-ß-lactamases and oxacillinase genes respectively. OXA-type carbapenemases have been divided into eight subgroups, with six identified in A. baumannii: OXA-23, OXA-40, OXA-51, OXA-58, OXA-143 and OXA-235 [5,6].

Overuse of carbapenems is an important factor for the development of colonization with carbapenem-resistant A. baumannii strains. The spread of carbapenem-resistant MDR A. baumannii has led to the use of polymyxins, particularly polymyxin E (colistin). Colistin, a polypeptide antibiotic, targets the bacterial outer membrane. Various mechanisms, including plasmid-mediated mcr-1 gene, mutations in the pmrA, pmrB, lpxA, lpxC and lpxD genes and the presence of insertion sequence ISAba1 in either lpxC or lpxA, are involved in the emergence of resistance to this antibiotic [7].

Aminoglycoside antibiotics have long been used for the therapy of infection in patients and are still a drug of choice for the treatment of diseases caused by MDR strains. Many researches show that A. baumannii can acquire drug resistance against these antibiotics [8]. Drug efflux pumps, methylases, 16S ribosomal RNA and AMEs play important roles in resistance to aminoglycosides. AMEs target different sites of these antibiotics [9,10]. The type of reaction assigns the nomination of AMEs, such as nucleotidyltransferases (ANT), phosphotransferase (APH) and acetyltransferase (AAC). The genes encoding AMEs may be located on class 1 integrons, transposons and plasmids in MDR A. baumannii [11].

The aims of this study were to investigate the frequency of the AME and oxacillinase genes among clinical isolates of A. baumannii recovered from hospitalized patients in Imam Khomeini Hospital in Ahvaz, Iran.

Materials and methods

Study design, data and specimens collection
This prospective cross-sectional study was performed between January 2018 and November 2019. Written informed consent was obtained from all patients (study approval IR.MU-BAM.REC.1398.072). The present research was carried out by using 80 strains of A. baumannii isolated from patients referred to Imam Khomeini Hospital in Ahvaz, south-west Iran. These strains were isolated from blood, urine, catheter, cerebrospinal fluid and trachea aspiration samples.

All samples were transported to the microbiology laboratory within 4 hours of collection and were processed immediately. In the following, the isolates were identified on the basis of standard bacteriologic tests, including Gram staining, oxidase, growth on MacConkey and blood agars, sugar fermentation on triple-sugar iron agar, methyl red, Voges-Proskauer, motility, Simmons citrate agar and urease [12]. Finally, confirmation was conducted by PCR of blaoXA-51-like gene and multiplex of gyrB [13]. The A. baumannii ATCC19606 strain was used as positive control.

Antimicrobial susceptibility testing
MICs of the following antibiotics was established via the VITEK 2 system (bioMérieux, Marcy l’Etoile, France). The antibiotics were as follows: ampicillin/sulbactam, piperacillin, cefepime, cefazidime, cefotaxime, amikacin, meropenem, imipenem, ciprofloxacin, levofloxacin, minocycline, gentamicin, tobramycin, tetracycline, colistin, tigecycline and trimethoprim/sulfa-methoxazole. The MICs of all the antibiotics were interpreted by the system according to Clinical and Laboratory Standards Institute guidelines [14].

A MIC ≥4 µg/mL for colistin was considered as the breakpoint of resistance as well as a MIC ≥8 µg/mL for imipenem and meropenem. Acinetobacter isolates are defined as MDR strains resistant to at least three classes of antimicrobial agents, including aminoglycosides, penicillins and cephalosporins and fluoroquinolones.

Molecular detection of oxacillinase and AME genes
Genomic DNA used as a template for PCR assays was extracted from isolates by boiling [15]. We investigated two groups of MDR A. baumannii genes: AME (aadA1, aadB, aphA6 and aacCI) and oxacillinases (blaoXA-23-like, blaoXA-24-like, blaoXA-51-like, blaoXA-58-like and blaoXA-143-like). The reactions were performed in 25 µL volume. The amplification mixture consisted of 200 µM deoxynucleotide triphosphates, 2.5 µL tenfold concentrate PCR buffer, 2.5 mM MgCl₂, 0.1 µM of each
primer, 1.5 U of Taq DNA polymerase (CinnaGen, Tehran, Iran); and finally 2 µL DNA was added to this mixture. Primer sequences used for the detection of these genes are presented in Table 1. The amplification conditions for oxacillinase genes were as follows: initial denaturation for 5 minutes at 94°C, 35 cycles of 94°C for 25 seconds, 52°C for 40 seconds and 72°C for 50 seconds. The final extension was performed at 72°C for 3 minutes. PCR conditions for AME genes included 35 cycles of amplification under the following conditions: denaturation at 95°C for 3 minutes; annealing at 53°C for 30 seconds; and final extension at 72°C, with a final extension at 72°C for 5 minutes. PCR products were resolved on 2.0% agarose gels, stained with safe stain and photographed by UV illumination.

Data analysis
The results were analysed by SPSS 22 (IBM, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) software. We considered p < 0.05 to be statistically significant, and 95% confidence intervals were used. The results are presented as descriptive statistics in terms of relative frequency.

**Results**

**Bacterial isolates**
A total of 80 *A. baumannii* strains were collected from the different clinical samples, including tracheal aspiration in 32 (36.25%), blood in 20 (25%), catheter in 16 (20%), urine in eight (10%) and cerebrospinal fluid in four (5%) isolates. Forty-six patients (57.5%) were male and 34 (42.5%) female; mean ± standard deviation age was 30 ± 1 years, with a range of 2 to 65 years. The frequency of collected *A. baumannii* isolates from different wards of the hospital was intensive care unit (ICU) in 31 (38.75%), urology in 20 (25%), general in ten (12.5%), surgery in six (7.5%), infectious diseases in seven (8.75%) and paediatric in six (7.5%) respectively (Table 2).

**Antibiotic susceptibility**
According to antibiotic susceptibility testing, among 80 *A. baumannii* strains screened, 75 isolates (91.25%) were MDR. The effect of the antimicrobial agents assessed in the present study against *A. baumannii* strains is listed in Table 3. The results showed that colistin and tigecycline, with respective sensitivity rates of 78 (97.5%) and 45 (56.25%), had the highest effect. Seventy-two isolates (90%) were resistant to gentamicin and amikacin simultaneously. More than 70% of the isolates were resistant to ciprofloxacin, amikacin, piperacillin, tetracycline, ampicillin/sulbactam, trimethoprim/sulfamethoxazole and gentamicin. Resistance to cephalosporins was higher than 90%. Also, 73 isolates (91.25%) and 64 isolates (80%) were resistant to imipenem and meropenem respectively. Sixty-three isolates (78.75%) were resistant to meropenem and imipenem simultaneously. The lowest level of MIC for imipenem was 0.5 µg/mL and the highest level was 512 µg/mL. Most of the meropenem-resistant isolates (29.68%) had meropenem MIC 16 µg/mL. Among strains sensitive to colistin, 13 isolates (16.25%) and two isolates (2.5%) had MIC 1 µg/mL and 2 µg/mL respectively. Two strains (Ab4 and Ab41) were resistant to colistin with MIC 32 and 16 µg/mL.

**Molecular characterization of oxacillinase**
The presence of *bla*OXA-1-like and *gyrB* genes was confirmed in all strains. Also, *bla*OXA-23-like and *bla*OXA-24-like genes were found in 68.75% (55/80) and 20% (16/80) respectively, while no isolate harbored the *bla*OXA-143-like gene. Also, the combination of *bla*OXA-23-like/*bla*OXA-24-like/*bla*OXA-51-like/*bla*OXA-58-like/*bla*OXA-23-like and *bla*OXA-24-like/*bla*OXA-23-like genes were seen in three (Ab8, Ab18 and

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**TABLE 1. Primers used for gene amplification**

| Target gene | Sequence (5’→3’) | Amplicon size (bp) | Reference |
|-------------|------------------|--------------------|-----------|
| *bla*OXA-1 | F-TAATGCTTTGATCGGCCCTTG | 353 | [16] |
| *bla*OXA-23 | R-TGGATTGCAGCTTGCATCGTG | 501 | [16] |
| *bla*OXA-24 | F-ATGGACACAACGCAGGTCGC | 246 | [16] |
| *bla*OXA-18 | R-GGTTAGTTGGCCCCCTTGGTG | 599 | [16] |
| *bla*OXA-1 | F-TGGACATTCTGCAACCTGCT | 149 | [17] |
| *bla*OXA-143 | R-TAATCTTGGAGGCGCCAACC | 797 | [18] |
| *apmA* | F-ATGGGACGCCCAATATTATTC | 465 | [18] |
| *aadC* | R-TCAATTCAATTTCAACTAATTTA | 534 | [18] |
| *aadB* | F-ATGGACACAACGCAGGTCGC | 792 | [18] |
| Strain Identification | Ward | Sample   | MER (µg/mL) | IMI (µg/mL) | COL (µg/mL) | OXA type | Gene Resistance to AMK/GEN |
|-----------------------|------|----------|-------------|-------------|-------------|----------|--------------------------|
| Ab1                   | General | TA      | 16          | 32          | 0.5         | 24       | A1 (+/+)                 |
| Ab2                   | ICU   | Blood   | 32          | 128         | 0.5         | 23       | B, A6 (+/+)              |
| Ab3                   | Urology | TA      | 64          | 16          | 0.5         | 23       | B, A6 (+/+)              |
| Ab4                   | ICU   | Catheter | 16          | 512         | 32          | 23       | A6 (+/+)                 |
| Ab5                   | Surgery | CSF    | 128         | 32          | 0.25        | 24       | A1 (+/+)                 |
| Ab6                   | Urology | TA      | 1           | 1           | 0.25        | 23, 24   | B (+/)                   |
| Ab7                   | ICU   | Blood   | 32          | 16          | 0.125       | 23       | C1 (+/)                  |
| Ab8                   | ICU   | Catheter | 16          | 128         | 0.5         | 23, 24, 5B | A6 (+/)                |
| Ab9                   | General | TA      | 32          | 64          | 1           | 23       | B, C1, A6 (+/)           |
| Ab10                  | Urology | Catheter | 64          | 128         | 1           | 23       | B (+/)                   |
| Ab11                  | ID     | TA      | 1           | 1           | 0.5         | 23       | C1 (+/)                  |
| Ab12                  | ICU   | Blood   | 16          | 128         | 0.25        | 23       | — (+/)                   |
| Ab13                  | Urology | Urine   | 32          | 32          | 0.5         | 24       | B (+/)                   |
| Ab14                  | Paediatric | TA    | 16          | 64          | 0.125       | 23       | A6 (+/+)                 |
| Ab15                  | ICU   | Blood   | 32          | 256         | 0.5         | 23       | C1 (+/+)                 |
| Ab16                  | General | TA      | 128         | 128         | 1           | 23       | A1 (+/+)                 |
| Ab17                  | Paediatric | Catheter | 1           | 0.5         | 0.5         | 23       | B (+/)                   |
| Ab18                  | Surgery | CSF    | 16          | 128         | 1           | 23, 24, 5B | C1 (+/+)                |
| Ab19                  | Urology | TA      | 64          | 16          | 1           | 23       | A6 (+/+)                 |
| Ab20                  | ICU   | Blood   | 0.5         | 256         | 0.5         | 23       | — (+/)                   |
| Ab21                  | ICU   | TA      | 32          | 64          | 0.5         | 23       | B, A6 (+/+)              |
| Ab22                  | ID     | TA      | 16          | 128         | 1           | 24       | A1 (+/+)                 |
| Ab23                  | Urology | Blood  | 64          | 32          | 0.25        | 23       | B (+/)                   |
| Ab24                  | General | Urine  | 2           | 256         | 0.25        | 24       | A6 (+/-)                 |
| Ab25                  | ICU   | Blood   | 32          | 128         | 0.5         | 23       | — (+/)                   |
| Ab26                  | Paediatric | TA    | 1           | 2           | 2           | 23, 24   | B, A6 (+/)              |
| Ab27                  | Paediatric | Urine | 64          | 64          | 1           | 23       | B (+/)                   |
| Ab28                  | ICU   | TA      | 16          | 32          | 0.5         | 24       | B, A6 (+/+)              |
| Ab29                  | ICU   | TA      | 64          | 32          | 0.5         | 23       | B (+/)                   |
| Ab30                  | Urology | Blood   | 0.5         | 256         | 0.25        | 23, 5B   | A1 (+/+)                 |
| Ab31                  | General | TA      | 64          | 128         | 0.125       | 23       | B (+/)                   |
| Ab32                  | ICU   | Catheter | 128         | 16          | 0.25        | 23       | A6 (+/+)                 |
| Ab33                  | Urology | Urine   | 16          | 128         | 0.25        | 24       | B (+/)                   |
| Ab34                  | Urology | Blood   | 2           | 64          | 1           | 23       | A6 (+/+)                 |
| Ab35                  | Surgery | TA      | 128         | 32          | 0.5         | 23       | — (+/)                   |
| Ab36                  | ICU   | Blood   | 32          | 128         | 2           | 23, 24   | B, A6 (+/+)              |
| Ab37                  | Paediatric | TA    | 128         | 16          | 0.25        | 23       | C1 (+/+)                 |
| Ab38                  | ID     | TA      | 64          | 128         | 0.5         | 24       | B (+/)                   |
| Ab39                  | ICU   | Catheter | 32          | 256         | 1           | 23       | A6 (+/+)                 |
| Ab40                  | Urology | TA      | 0.5         | 32          | 0.25        | 23       | B (+/)                   |
| Ab41                  | ICU   | Blood   | 128         | 256         | 16          | 23       | A6 (+/+)                 |
| Ab42                  | ICU   | TA      | 16          | 32          | 0.25        | 23       | B (+/)                   |
| Ab43                  | General | Catheter | 64          | 64          | 0.25        | 24       | B (+/)                   |
| Ab44                  | Urology | Blood   | 32          | 32          | 0.5         | 23       | A1 (+/+)                 |
| Ab45                  | ICU   | TA      | 64          | 256         | 0.25        | 23       | A6 (+/+)                 |
| Ab46                  | ID     | Catheter | 0.5         | 1           | 0.5         | 23       | C1 (+/+)                 |
| Ab47                  | ICU   | TA      | 16          | 128         | 0.125       | 24       | B (+/)                   |
| Ab48                  | Urology | TA      | 128         | 256         | 0.25        | 23       | C1 (+/+)                 |
| Ab49                  | Surgery | Blood   | 64          | 32          | 0.5         | 23       | B (+/)                   |
| Ab50                  | ICU   | CSF     | 16          | 16          | 0.5         | 23       | B, A6 (+/+)              |
| Ab51                  | ID     | Blood   | 128         | 32          | 0.5         | 23       | A6 (+/+)                 |
| Ab52                  | ICU   | CSF     | 256         | 64          | 1           | 23, 24   | C1 (+/+)                 |
| Ab53                  | General | TA      | 16          | 128         | 0.25        | 24       | A6 (+/+)                 |
| Ab54                  | Urology | Catheter | 16          | 32          | 0.5         | 23       | — (+/)                   |
| Ab55                  | ICU   | Urine   | 128         | 32          | 0.5         | 23       | A1 (+/+)                 |
| Ab56                  | Paediatric | TA    | 256         | 16          | 0.25        | 23       | B (+/)                   |
| Ab57                  | ICU   | Blood   | 0.5         | 128         | 0.25        | 23, 5B   | A6 (+/+)                 |
| Ab58                  | ICU   | Catheter | 32          | 64          | 0.25        | 24       | B (+/)                   |
| Ab59                  | Urology | TA      | 32          | 128         | 0.5         | 23       | B (+/)                   |
| Ab60                  | ID     | TA      | 128         | 256         | 0.25        | 23, 24   | B (+/)                   |

Gene Resistance to AMK/GEN: NMNI, COL, GEN, tigecycline; A, medium; B, weak; XDR, extensively drug resistant.

A1, aadA1; A6, aphA6; AMK, amikacin; B, aadA1; C1, aacC1; COL, colistin; GEN, gentamicin; ID, infectious disease; M, medium; MDR, multidrug resistant; MER, meropenem; N, non-β-lactamase producing; PF, pleural fluid; S, strong; TA, tracheal aspirate; TGC, tigecycline; W, weak; XDR, extensively drug resistant.
Ab60) and four (Ab6, Ab26, AB36 and Ab52) of the isolates respectively. The coexistence of the bla_{OXA-58}-like and bla_{OXA-23}-like genes occurred in two isolates (Ab30 and Ab57). In isolates with the bla_{OXA-24}-like and bla_{OXA-23}-like genes, 68.75% (11/80) and 47.27% (26/80) of isolates had imipenem MICs of 64 μg/mL or more respectively. In isolates with bla_{OXA-24}-like and bla_{OXA-23}-like genes, 31.25% (5/16) and 40% (22/55) had meropenem MICs of 64 μg/mL or more respectively (Table 4).

AME genes

All strains were screened for the presence of genes encoding the AMEs. Screening of these genes by PCR showed that frequency of aadA1, aacC1, aphA6 and aadB were 11.25% (9/80), 16.25% (13/80), 22.5% (18/80) and 30% (24/80) respectively. Two strains (7.5%) had aadB, aacC1 and aphA6 genes, and six strains had aadB and aphA6 genes. Also, eight strains did not have any AME genes. All strains with two or three AME genes were 100% resistant to gentamicin and amikacin. In addition, resistance to these antibiotics in strains with one AME gene was more than 60%. Analysis of coexistence of AME and genes encoding OXA carbapenemases indicated that more than 50% of strains with AME genes had bla_{OXA-23}-like genes (Table 4). In the four isolates that were resistant to gentamicin and amikacin, no AME genes were detected.

**Discussion**

Nosocomial infections caused by MDR A. baumannii are a major problem worldwide, particularly in ICUs [19]. Our results shed light on the pressing issue of antimicrobial resistance among MDR A. baumannii isolates in Ahvaz, south-west Iran. In the present study, more than 38% of A. baumannii strains were isolated from samples obtained from patients in the ICU. As has been demonstrated, ICUs are high-risk areas for nosocomial infections because of the severity of underlying diseases, the use of invasive procedures and the duration of stay [20].

One of the main concerns in the treatment of Acinetobacter infections is the emergence of MDR and pandrug-resistant Acinetobacter isolates. Some outbreaks related to MDR Acinetobacter spp. have been recently described in several countries [21–23]. In the current study, we observed a high prevalence of the MDR phenotype (75/80, 91.25%) in A. baumannii isolates, a finding in agreement with other studies [24,25].

Carbapenems have become the favoured treatment for serious Acinetobacter infections in many centres and have retained better activity than other antimicrobial agents [26]. These antimicrobials have been utilized in Iran in most recent years, yet there is some evidence that increased and uncontrolled use of these agents favours the emergence of resistance to carbapenems [19]. Although we only tested susceptibility to imipenem and meropenem, we observed species that were highly resistant to carbapenems in this investigation, of which 91.2% were resistant to imipenem and 80% to meropenem. In a systematic review, the antimicrobial resistance rate of A. baumannii was assessed among 3409 samples collected from Iranian patients from 2001 to 2013 (51.1% imipenem, 64.3% meropenem) [27].

The investigation indicated a noteworthy increase in resistance rate against imipenem and meropenem, while resistance to aminoglycosides and lipopeptides did not have markedly change during these years. Resistance to carbapenems at the start of the research in 2001 was low (64.3% for meropenem and 51.1% for imipenem), but by the study’s end in 2013, it reached 81.5% for meropenem and 76.5% for imipenem. Because of the high rate of A. baumannii resistance to studied antibiotics, they cannot be used as empirical treatment. Therefore, several studies have researched combination therapy of two or more agents for MDR Acinetobacter infections [28]. Recently tigecycline and colistin have developed as an alternative treatment choice for MDR Acinetobacter infections [29]. As shown in Table 3, colistin was the most active antimicrobial agent against most isolates of A. baumannii, with a susceptibility rate of 97.5%.

Because the frequency of resistance to colistin is low, it tends to be utilized as an easily accessible antibiotic for treatment of MDR A. baumannii strains susceptible to this agent [28,30]. In an investigation of 91 A. baumannii isolates from patients in tertiary-care ICUs of three university hospitals in

**TABLE 3. Antibiotic susceptibility pattern of Acinetobacter baumannii isolates**

| Susceptibility pattern | COL  | TGC  | IMI  | MER  | CTZ  | DFP  | CTX  | CIP  | TET  | PIP  | SXT  | AMP/S | AMK  | GEN  |
|------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Resistant              | 71   | 67   | 63   | 76   | 77   | 55   | 74   | 78   | 80   | 75   | 64   | 73   | 35   | 2    |
| Intermediate           | 0    | 0    | 0    | 2    | 2    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Susceptible            | 9    | 13   | 17   | 21   | 23   | 15   | 25   | 30   | 35   | 45   | 58   | 74   | 100  | 0    |

Data are presented as n (%). AMK, amikacin; AMP/S, ampicillin/sulbactam; CFP, cepofaxime; CIP, ciprofloxacine; COL, colistin; CTX, cefotaxime; CTZ, cefazidime; GEN, gentamicin; GEN, gentamicin; IMI, imipenem; LEV, levofloxacine; MEM, meropenem; MIN, minocycline; PIP, piperacillin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TOB, tobramycin.

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Analysis of occurrence of aminoglycoside resistance genotypes, resistance patterns to amikacin (AMK), gentamicin (GEN) and genes encoding OXA carbapenemases among Acinetobacter baumannii strains

| AME gene | N (%) | Resistance to AMK | Resistance to GEN | Resistance to AMK, GEN | blaOXA-23-like | blaOXA-24-like |
|----------|-------|-------------------|-------------------|------------------------|---------------|---------------|
| aphA6    | 18 (22.5) | 15 (83.3) | 18 (100) | 15 (83.3) | 14 (77.7) | 2 (11.1) |
| aadA      | 9 (11.25) | 6 (66.6) | 8 (88.8) | 6 (66.6) | 5 (55.5) | 3 (33.3) |
| aacC1     | 24 (30) | 21 (87.5) | 22 (91.6) | 21 (87.5) | 14 (58.3) | 7 (29.1) |
| aacC1, aphA6 | 13 (16.25) | 10 (76.9) | 10 (76.9) | 8 (61.5) | 9 (69.2) | 2 (15.2) |
| aadB      | 6 (7.5) | 6 (100) | 6 (100) | 6 (100) | 4 (66.6) | 1 (16.6) |
| aadB, aacC1, aphA6 | 2 (2.5) | 2 (100) | 2 (100) | 2 (100) | 2 (100.0) | 0 (0.0) |
| Negative  | 8 (10) | 7 (87.5) | 5 (62.5) | 4 (50) | 7 (87.5) | 1 (12.5) |
| Total     | 67% | 71% | 62% | 55% | 16% |

Data are presented as n (%) unless otherwise indicated. AME, aminoglycoside-modifying enzyme; CSF, cerebrospinal fluid.

In agreement with our results, a study from Tehran noted the coexistence of blaOXA-23-like/blaOXA-58-like genes in two isolates (3%) [46]. In another study, the blaOXA-58-like gene was identified in 30.95% of the studied isolates—remarkably higher than our study [47]. Also, in agreement with the current study regarding coexistence, Alavi-Moghaddam et al. [48], Tafreshi et al. [47] and Pournajaf et al. [45] reported coexistence rates of 12.1%, 3.57% and 17.4% respectively for blaOXA-23-like and blaOXA-24-like genes in their isolates.

Aminoglycosides are generally utilized for the treatment of Acinetobacter infection in Iran; however, at present, the increasing emergence of highly resistant strains is causing major concerns. Overall susceptibility rates to amikacin and gentamicin were 16.2% and 11.2% respectively. According to the molecular analysis of aminoglycoside-resistant strains, AMEs have been proposed as the principal mechanism related to aminoglycoside resistance [49].

The presence of highly frequent aadA1 (11.25%), aacC1 (16.25%) aphA6 (22.5%) and aadB (30%) genes were demonstrated on clinical isolates of Acinetobacter by PCR. In a study from Tehran, screening of AME genes showed that frequency of aadB, aphA6, aadA1 and aacC1 genes was 72%, 65%, 37% and 21% respectively [50]. Gholami et al. [51] indicated that the aac(6’)-Ib, aac(3)-I, aphi(3’)-I and armA genes are more prevalent than other genes; aac(6’)-Id and mmtA genes were found only at a very low incidence in the tested isolates. A study conducted by Xiao et al. [52] on A. baumannii isolates showed that the prevalence of aac(3)-I, aac(6’)-Ib, ant(2’)-I and aphi(3’)-I genes were 10.7%, 17%, 14.3% and 17% respectively, and except for armA (17.9%), other types of 16S ribosomal RNA methylase genes were not detected in any of the isolates.

Conclusion

Our findings indicate that the presence of the aadB and aphA6 genes was correlated with high resistance against amikacin and gentamicin. We also found a very high resistance rate against...
most of the antimicrobial agents usually prescribed to treat infections. Monotherapy should be avoided because of the rapid emergence of resistance, even with colistin or tigecycline. Strict infection control strategies and antimicrobial resistance surveillance programmes are still lacking in Iran despite the alarming emergence of MDR A. baumannii, especially among colistin-resistant isolates. We hope these results change the attitude of physicians regarding utilizing antibiotics in ICUs; we encourage them to follow antibiotic stewardship guidelines as the only effective strategy to (somewhat) control antibiotic resistance in healthcare settings.

Conflict of interest

None declared.

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