Carbapenem resistance in Acinetobacter baumannii clinical isolates from northwest Iran: high prevalence of OXA genes in sync

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ABSTRACT

Background and Objectives: Carbapenem treatment for Acinetobacter baumannii infections presently faces threats owing to the production of several types of carbapenemase enzymes, prevalence of which varies among different countries. We explored the current trend of antibiotic resistance in A. baumannii clinical isolates from North West Iran, sought the mechanism of carbapenem resistance and addressed the sequence type groups in carbapenem resistant A. baumannii (CRAB).

Materials and Methods: A. baumannii (n=112) isolates were recovered from various clinical specimens of patients admitted in internal, surgery, burn, infectious diseases and various ICUs wards. Genetically confirmed A. baumannii isolates were screened for carbapenem resistance by the Kirby-Bauer and E-test and the presence of blaBL, blaOXA-like, ISAba1 genes by PCR. Sequence groups were identified by multiplex PCR.

Results: Multidrug-resistance (MDR) was a characteristic feature of all A. baumannii isolates. Frequency of oxacillinase genes in combination including blaOXA-51-like, blaOXA-23-like, blaOXA-51-like, blaOXA-24-like and blaOXA-23-like, blaOXA-24-like was 82.1%, 36.6% and 25.8% respectively. Blending of oxacillinase and MBL genes was evident in eight blaOXA-23-like positive and 7 blaOXA-24-like positive isolates thereby depicting synchronous etiology of carbapenem resistance. Amongst CRAB isolates, 97.3% contained ISAba1 element and 50.9% belonged to the European clone II.

Conclusion: Synchronicity among blaOXA-like with blaBL and ISAba1 gene was a hallmark of this investigation. Though origin or route of transmission was not elucidated in this study but co-existence among OXA and MBL producing genes is a therapeutic concern demanding strict surveillance strategies and control programs to halt the dissemination of these isolates in the hospital setting.

Keywords: Acinetobacter baumannii; Carbapenems; Carbapenemase; Oxacillinase; Beta-lactamase; Drug resistance; Multiplex polymerase chain reaction
INTRODUCTION

Emergence of Multi Drug-Resistant (MDR) and Extensive Drug-Resistant (XDR) *Acinetobacter baumannii* (*A. baumannii*) strains has complicated the therapeutic regime to treat the infections caused by the organism thereby authenticating “turning old friend into an enemy” with certainty (1). Carbapenems that were once the drug of choice has now been almost contemplated as an outgoing treatment due to the emergence of Carbapenem Resistant *A. baumannii* (CRAB). This remarkable aptitude of *A. baumannii* to gain antibiotic resistance has clasped the infectious specialist’s hands (1, 2).

Production of Ambler class B Metallo β-Lactamases (MBLs) and Ambler class D oxacillinas (known as Carbapenem Hydrolyzing class D β-Lactamases or CHDLs), are the two main contributors of carbapenem resistance in *A. baumannii*. Many MBLs implicated in CRAB encompass bla<sub>IMP</sub>, bla<sub>NDM</sub>, bla<sub>VIM</sub>, bla<sub>IMP</sub>, bla<sub>SPM</sub> and bla<sub>NDM</sub> genes, the prevalence of which varies depending upon the various geographical locations (1-4). At present six groups of CHDLs have been described in *A. baumannii* such as bla<sub>OXA-23-like</sub>, bla<sub>OXA-24/40-like</sub>, bla<sub>OXA-51-like</sub>, bla<sub>OXA-181-like</sub>, and bla<sub>OXA-235-like</sub> that are consistently associated with resistance or at least with reduced susceptibility of *A. baumannii* towards carbapenems (5-7). Although bla<sub>OXA-51-like</sub> weakly hydrolyze carbapenems, but can confer high resistance when overexpressed because of association with mobile genetic elements (MGEs). These elements contain strong promoters that play a major role in the expression of antibiotic resistance genes located downstream from the insertion site of these mobile elements (1, 8). *A. baumannii* intrinsically carry bla<sub>OXA-51-like</sub> gene, which encodes for a weak carbapenemase production. Presence of an upstream ISA<sub>β</sub>al-β gene enhances the level of expression of this carbapenemase and has been reported as the promoter for bla<sub>OXA-51-like</sub> and probably, for bla<sub>OXA-23-like</sub> carbapenemase genes (1, 2, 8).

Many genomic fingerprinting methods utilized to determine epidemiologic links and molecular relatedness of the isolates include Repetitive Extragenic Palindromic-Polymerease Chain Reaction (REP-PCR), Pulsed-Field Gel Electrophoresis (PFGE), Matrix-Assisted Laser Desorption/Ionization Time Of Flight (MALDI-TOF), Mass Spectrometry (MS), Multilocus Sequence Typing (MLST), Amplified Fragment Length Polymorphism (AFLP), Amplified Ribosomal DNA Restriction Analysis (ARDRA), Randomly Amplified Polymorphic DNA Analysis (RAPD), RNA spacer fingerprinting, and sequence analysis of 16S-23S rRNA with various advantages and disadvantages. Intergene spacer regions or the gyrB and rpoB genes and multiplex PCRs for major international clone/sequence groups (SGs) have been established and fully vetted to investigate the clonal spread (4). SGs typing is less laborious, reproducible, cost effective and facile technique. Moreover, this technique facilitates rapid identification of the sequence type group or clonal lineage of outbreak strains, without any need for sequencing or other typing techniques. This approach has been proved helpful in identifying the genotypes that are most likely to cause of infection in hospitals (9).

The last few years have witnessed a steep increase in CRAB phenotype in our hospital. As our hospital is one of the referral hospitals for the Northwest of Iran especially for burn patients, emergence of CRAB strains is a medical concern. This study aimed to investigate the molecular mechanisms involved in carbapenem resistance in *A. baumannii* obtained from various infections in a hospitalized patients and probe the sequence groups among these CRAB isolates.

MATERIALS AND METHODS

**Bacterial isolates.** Between October 2018 to October 2019, 112 *A. baumannii* isolates were collected from Tabriz University of Medical Sciences based-Sina Educational, Treatment and Research Hospital. The source of these isolates comprised endotracheal aspirate (n=32), wound (n=38), blood (n=22), urine (n=12), Broncho-alveolar lavage (n=5) and IV catheter (n=3) from patients admitted in internal, surgery, burn, infectious diseases wards and various ICUs. The clinical specimens were cultured on blood agar and MacConkey media (Liofilchem, Italy) and the suspected *A. baumannii* colonies were initially identified by standard biochemical methods (10). Identification of *A. baumannii* was confirmed by the amplification of DNA gyrase subunit B (gyrB) and RNA polymerase β subunit (rpoB) genes using PCR as described earlier (11, 12) and eventually isolates were preserved at -70°C in Trypticase Soy Broth (TSB) (Liofilchem, Italy) containing 20% (v/v) glycerol, for
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This study was approved by Ethical Committee of Tabriz University of Medical Sciences, [IR.TBZMED.REC.1397.042].

**Antimicrobial susceptibility testing.** Initial antimicrobial susceptibilities were performed using (Kirby-Bauer) disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI-2018) guidelines and results interpreted accordingly (13). The antibiotics tested were: imipenem (10 μg), meropenem (10 μg), doripenem (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), piperacillin/tazobactam (100/10 μg), and ampicillin/sulbactam (10/10 μg) (Liofilchem, Italy). *A. baumannii* isolates were defined as MDR and XDR phenotypes according to the International Expert Proposal for Interim Standards Guidelines (14). By definition, strains resistant to at least one antibacterial agent in three or more antimicrobial categories, were classified as MDR strains and those MDR strains that showed resistance to at least one agent in all antimicrobial classes but remain susceptible to only one or two antimicrobial categories, were classified as XDR strains (14).

The Minimum Inhibitory Concentrations (MICs) of carbapenems was determined for all *A. baumannii* isolates using E-test strips (imipenem, meropenem and doripenem) (Liofilchem, Italy) according to the manufacturer’s instructions. Briefly, the strips were placed onto Mueller Hinton agar plates (Liofilchem, Italy) that had been inoculated with a bacterial suspension equivalent to 0.5 McFarland and incubated at 35°C. The results of imipenem, meropenem and doripenem were interpreted according to (CLSI-2018) (13). MICs of colistin was determined by broth dilution method in Mueller-Hinton broth according to the (CLSI-2018) guidelines (13). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

**Phenotypic detection of MBLs.** All CRAB isolates were screened for MBL production using MBL E-test strips (Liofilchem, Italy) according to the manufacturer's instructions. These E-test strips contained imipenem (IMI: 4-256 μg/mL) and imipenem + EDTA (IMD: 1-64 μg/mL). The strain was considered as MBL producer when the IMI/IMD ratio was ≥ 8 μg/mL, or the presence of a phantom zone, means an extra inhibition zone between the IMI and IMD regions (15).

**Detection of carbapenemase genes.** The prevalence of different carbapenemase genes among CRAB was performed by conventional PCR. DNA template was prepared using QIAamp DNA Mini kit (Qiagen GmbH, Germany). Screening CHDL genes was achieved by performing multiplex PCR including bla<sub>OXA-51-like</sub>, bla<sub>OXA-23-like</sub>, bla<sub>OXA-24/40-like</sub> and bla<sub>OXA-58-like</sub> using primers described elsewhere (Supplementary Table). Presence of MBL genes was performed by two multiplex PCRs including bla<sub>IMP</sub>, bla<sub>CM</sub>, bla<sub>SIM</sub> and bla<sub>GIM</sub> using specific primers as previously described (6, 7, 16) and (17, 18) respectively (Supplementary Table). Insertion sequence was detected in *A. baumannii* isolates using ISAba1 specific forward and reverse primers (ISAba1-F/ISAba1-R) as described previously (8) (Supplementary Table 1). For detection of ISAba1 linkage PCR was performed using the ISAba1 forward primer in combination with bla<sub>OXA-23-like</sub> or bla<sub>OXA-51-like</sub> reverse primers as described elsewhere (8, 16). *A. baumannii* reference strains NCTC 13304 and NCTC 13302 were used as positive controls for the amplification of bla<sub>OXA-23-like</sub> or bla<sub>OXA-51-like</sub> genes.

**Identification of sequence groups (SGs).** To determine the clonal lineages of *A. baumannii*, two groups of primers were used for performing two multiplex PCRs, which revealed international clonal lineages. Multiplex PCRs were performed to selectively amplify SG1 and SG2 alleles of the gene encoding outer-membrane protein A (*ompA*), the gene encoding part of a pilus assembly system required for biofilm formation (*csuE*) and the intrinsic bla<sub>OXA-51-like</sub> carbapenemase gene of *A. baumannii* as described previously (1, 9). Identification of a strain as SG1 (EC II: European Clone II) and SG2 (EC I: European Clone I) or other new groups of SGs, was performed according to previously described study (9).

**Statistical analysis.** The chi square or Fisher’s exact test compared categorical variables using SPSS 22.0 statistical software (SPSS Inc. Chicago, IL). Variables with a P value of ≤ 0.05 were included in the final analysis. Cross tabulation was done and Sensitivity (SN), Specificity (SP), Positive Predictive
Supplementary Table 1. Primer sequences used in the study

| Primers          | Sequence (5’-3’)                      | Product size | References |
|------------------|---------------------------------------|--------------|------------|
| gyrB- (Sp4-F)    | CAGCCCGTAAAGAGTAGCATTA                | 294 bp       | 11         |
| gyrB- (Sp4-R)    | AACGGAGCTTGCAGGGGTTA                 | 490 bp       | 11         |
| gyrB- (Sp2-F)    | GTTCTGATCCGAAATTTCCTCG               | 350 bp       | 12         |
| rpoB-F           | TAYCGYAAAGAYTTGAAAGAAGG               | 501 bp       | 16         |
| rpoB-R           | CMACACCCYTTGTTMCCRTGA                | 246 bp       | 16         |
| bla (OXA-23/41)  | GATCGGATGGAAGAACCAGA                 | 149 bp       | 6          |
| bla (OXA-23/41-R)| GATCGGATGGAAGAACCAGA                 | 149 bp       | 6          |
| ISAba1-F         | CCAATGTCAGAGGTTG                     | 768 bp       | 7          |
| ISAba1-R         | CGACGAAATCTAGCAC                     | 549 bp       | 8          |
| blaNM-F          | GGAATGAGTGGCTAATTAATCT               | 188 bp       | 17         |
| blaNM-R          | CAAATCAGGTGAAAGAAGG                  | 390 bp       | 17         |
| blaVRE-F         | CAAATCAGGTGAAAGAAGG                  | 570 bp       | 17         |
| blaVRE-R         | CAAATCAGGTGAAAGAAGG                  | 477 bp       | 17         |
| blaCM-F          | CAAATCAGGTGAAAGAAGG                  | 271 bp       | 17         |
| blaCM-R          | CAAATCAGGTGAAAGAAGG                  | 621 bp       | 18         |

Values (PPV) and Negative Predictive Values (NPV) of phenotypic test was calculated for carbapenem resistant isolates against blaMBL genes. PCR was considered as the gold standard.

RESULTS

Bacterial source. Majority (86.6%) of A. baumannii isolates were recovered from patients admitted in various ICU wards including burn ICU (26.8%), internal ICU (19.6%), infectious diseases ICU (16.1%), general ICU (16.1%), surgical ICU (5.4%) and toxicology ICU (2.7%) while, 13.4% isolates belonged to in-patients admitted to other wards. These specimens were obtained from patients in different ages and the range included from 5 to 85 years old (Mean ± SE = 56.8 ± 1.37).

Antimicrobial susceptibility pattern by disk diffusion. All 112 isolates were resistant to cefotaxime, ceftazidine, ceftriaxone, imipenem, meropenem, doripenem, ciprofloxacin, levofloxacin, piperacillin/tazobactam and co-trimoxazole while, moderate susceptibility was noticed towards ampicillin-subbac- tam (51.8%), tobramycin (35.7%), gentamicin and amikacin (27.7%). All A. baumannii isolates were MDR (100%) and among these, 51.8% isolates were
resistant to all classes of antibiotics except colistin and ampicillin-sulbactam, thus were classified as XDR phenotypes (Table 1).

**MICs of carbapenems and colistin.** MICs of imipenem, meropenem and doripenem was > 32 μg/mL for all phenotypically determined carbapenem resistant A. baumannii isolates. Detection of MICs for colistin in CRAB isolates by micro broth dilution method indicated that all A. baumannii isolates were susceptible to colistin (MIC < 2 μg/mL). Results of disk diffusion for carbapenems were compatible with MICs.

**Prevalence of MBL producers.** The phenotypic detection of MBL producing strains using imipenem/imipenem+EDTA E-test strips was noticeable in 33.9% (n=38) of A. baumannii isolates. Though this method was associated with 100% sensitivity, 74% specificity, however, high number of false positive results and low Positive Predictive Values (PPV=31%) indicate that these methods may not be suitable for detection of MBL producer strains.

**Prevalence of MBL genes.** All A. baumannii isolates were examined for six MBL encoding genes whereby 12 (10.7%) isolates were positive for the MBL genes, the most frequent being blaoxaNDM, 6.2% (n=7), followed by blamim, 4.4% (n=5) while, other tested MBL genes (blavIMP, blasm, blagIM, and blasPA) were not detected in any isolate.

**Prevalence of the blaoxa-1bla encoding genes.** All CRAB isolates carried the naturally occurring intrinsic blaoxa-51- bla gene, 82.1% (n=92) isolates were positive for blaoxa-244-1bla and 36.6% (n=41) harbored blaoxa-1bla genes. Coexistence of three different blaoxa-1bla genes (blaoxa-244-1bla, blaoxa-51-1bla and blaoxa-2480-1bla) was a prominent feature in 25.8% (n=29) isolates. Combination of different blaoxa-1bla and blambl genes (blaoxa-244-1bla, blaoxaNDM, blambl) and (blaoxa-2440-1bla, blaoxaNDM, blambl) was detected in 71% (n=8) and 6.2% (n=7) isolates, respectively (Figs. 1 and 2). Furthermore, 4.4% (n=5) isolates were positive for only blaoxa-51-bla gene, lacking other blaoxa-1bla or blambl genes. None of the A. baumannii isolate was positive for blaoxa-51-bla, blaoxa-244-1bla, and blaoxa-235-1bla genes (Fig. 1).

**Prevalence of ISAba1 upstream of blaoxa-244-1bla gene.** ISAba1 element was found in 95.5% (n=107) A. baumannii isolates. In sixty-four (69.5%) isolates with blaoxa-244-1bla, ISAba1 lay upstream of blaoxa-244-1bla however, ISAba1 was not detected upstream of blaoxa-51-1bla gene (Fig. 2).

**International clonal lineages.** Multiplex PCR for the identification of SGs revealed 50.8% (n=57) A. baumannii isolates belonged to SG1 (EC II). Among these, 93% (n=53) isolates were recovered from ICU patients and the source of 36.8% (n=21) was found as endotracheal aspirate whereas, 6.2% (n=7) isolates belonged to the SG2 (EC I) and 5.3% (n=6) belonged to the SG3 (EC III). All SG2 and SG3 isolates were recovered from ICU patients. The source of four isolates each in SG2 and SG3 groups was wound (Fig. 3). Furthermore, 37.5% (n=42) isolates belonged to new variants of SGs. These variants included 28.5% (n=12) SG4, 19% (n=8) SG5, 23.8% (n=10) SG6, 14.2% (n=6) SG7, 9.5% (n=4) SG8 and 4.7% (n=2) SG9. Fig. 4 depicts the distribution of OXA and MBL genes in three major sequence groups. No significant difference was evident in the frequency of oxacillinase and MBL genes in the sequence groups.

Table 1. Antimicrobial resistance patterns of carbapenem resistant A. baumannii

| Antimicrobial resistance profile* | Number (%) |
|---------------------------------|------------|
| IMI, MRP, DOR, CAZ, CTX, CRO, CIP, LEV, SXT, PTZ | 100 (112) |
| IMI, MRP, DOR, CAZ, CTX, CRO, CIP, LEV, SXT, PTZ, GM, AK | 72.3 (81) |
| IMI, MRP, DOR, CAZ, CTX, CRO, CIP, LEV, SXT, PTZ, GM, AK, TOB | 64.2 (72) |
| IMI, MRP, DOR, CAZ, CTX, CRO, CIP, LEV, SXT, PTZ, GM, AK, TOB, AMS | 48.2 (54) |
| IMI, MRP, DOR, CAZ, CTX, CRO, CIP, LEV, SXT, PTZ, GM, AK, TOB, AMS | 0 (0) |

*IMI: Imipenem, MRP: Meropenem, DOR: Doripenem, CAZ: Cefazidime, CTX: Cefotaxime, CRO: Ceftriaxone, CIP: Ciprofloxacin, LEV: Levofloxacin, SXT: Co-trimoxazole, PTZ: Piperacillin/tazobactam, GM: Gentamicin, AK: Amikacin, TOB: Tobramycin, AMS: Ampicillin/sulbactam
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Fig. 1. PCR analysis depicting blaOXA-like encoding genes

Line 1: Clinical isolate showing blaOXA-51-like (353 bp)
Line 2, 3, 7: Clinical isolates depicting blaOXA-23-like (501 bp) and blaOXA-51-like (353 bp)
Line 4, 5, 6: Clinical isolates depicting blaOXA-23-like (501 bp), blaOXA-51-like (353 bp) and blaOXA-24-like (246 bp)
Line 8: A. baumannii reference strain NCTC 13304 (positive control) showing blaOXA-51-like (353 bp) and blaOXA-23-like
Line 9: A. baumannii reference strain NCTC 13302 (positive control) showing blaOXA-24-like and blaOXA-51-like (353 bp)
Line 10: Size marker (100 bp DNA Ladder)

Fig. 2. Prevalence of OXA, MBL and ISAbl genes in A. baumannii clinical isolates

a) Prevalence of oxacillinase and metallo-β-lactamase genes
b) Prevalence of ISAbl and ISAbl upstream of oxacillinase genes
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Fig. 3. Source of *A. baumannii* clinical isolates in three sequence groups

a) Distribution of SG1 (EU II) in clinical specimens
b) Distribution of SG2 (EU I) in clinical specimens
c) Distribution of SG3 (EU III) in clinical specimens

DISCUSSION

*Acinetobacter baumannii* is one of the most important bacteria in ICUs, because of its remarkable ability to acquire antibiotic resistance and adaptability to survive in hospital environment (1, 4). Since last eight years an escalating frequency of *A. baumannii* in the ICUs has been reported from studies performed at various regions of Iran whereby the prevalence was reported as 37%, 59.3% and 74.2%, respectively (15, 19, 20). Higher prevalence of *A. baumannii* in ICUs in our study can be due to the specimens obtained only from hospital setting. The Sina hospital is a referral center receiving burns patients from the entire Northwest region. Prevalence of *A. baumannii* in patients admitted to ICUs even varies among different countries and range from 28% to 69.2% (21-24).

Among the various clinical specimens, the highest
number of *A. baumannii* isolates were from wound and endotracheal aspirate specimens (n=38 and n=32, respectively). High prevalence of *A. baumannii* from wound (mostly burn wound specimens) in our study was predictable with regard to burn and burn ICU wards in this hospital. Compatible results are available from study conducted earlier in Iran (20, 25), and elsewhere in India (21) and Saudi Arabia (22), whereby the prevalence has been reported as 28% and 22%, respectively. High prevalence of *A. baumannii* in endotracheal aspirate specimens in the present research can be partly explained by the fact that *A. baumannii* is the most frequent pathogen causing respiratory tract infections especially in ICUs patients. Nevertheless, our results show lower prevalence compared to similar studies conducted in India (21), Turkey (23) and Saudi Arabia (22), whereby prevalence of 31%, 54% and 31.5% has been reported respectively.

In the present investigation, all *A. baumannii* isolates were resistant to carbapenems, cephalosporins, fluoroquinolones and co-trimoxazole. This rate of antibiotic resistance is higher than studies conducted previously in Iran (15, 19), whereby carbapenem (imipenem/meropenem) resistance varied from 62% and 78% along with variable results of high resistance concerning to other antibiotics such as third generation cephalosporins and fluoroquinolones. In the present study, all *A. baumannii* isolates belonged to MDR phenotype. Of these isolates, 51.8% were XDR phenotype based on the resistance to all except one or two class of antibiotics (only susceptible to colistin and ampicillin-sulbactam) in this study. The rate of MDR *A. baumannii* in previous studies have been reported to vary from 59% to 100% in studies conducted in Iran (Tehran), India, Kuwait and Spain (20, 21, 26, 27).

Carbapenem resistance was confirmed by the MICs results in the present study and unusual high-level resistance to imipenem, meropenem and doripenem (MIC > 32 µg/mL) was displayed. There was no discrepancy between the rate of resistance to carbapenems by the disk diffusion and E-test method. High rate of carbapenem (imipenem/meropenem) resistance has been witnessed earlier in Iranian research ranging from 62% to 85% (15, 19, 28-30). Compatible rate of carbapenem resistance has been evidenced from other countries (21, 24, 26, 31), which is an indication that this increase is a global upsurge.

Carbapenem resistance is a considerable concern

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**Fig. 4.** Prevalence of carbapenemase genes in three sequence groups

a) Prevalence of OXA, MBL genes and ISAba1 in SG1 (EUII)

b) Prevalence of OXA, MBL genes and ISAba1 in SG2 (EUI)

c) Prevalence of OXA, MBL genes and ISAba1 in SG3 (EUIII)
as these antibiotics were the last resort of therapeu-
tic regimen until recently for the treatment of serious
nosocomial infections caused by A. baumannii. With
the emergence of high-level carbapenem resistance,
treatment of infections caused by A. baumannii is a
challenge. It seems that the emergence of CRAB
strains in the world may be due to the extensively
overuse or non-judicious use of these antibiotics
among hospitalized patients (2, 4).

Despite the high level of resistance to almost all
antibiotics in our study, colistin retained its effica-
cy against A. baumannii with susceptibility rate of
100%. Though this finding is consistent with most
studies conducted earlier in Iran (15, 28, 30) never-
theless, resistance to colistin has been on increase in
studies conducted in Iran and other parts of the world
(India, Saudi Arabia, Kuwait) (20-22, 26). Colistin is
one of the last options for the treatment of CRAB in-
fecteds and as evidenced there is tendency that with
overuse its resistance rate may increase. Indeed, A.
baumannii isolates resistant to carbapenem and colis-
tin simultaneously have been identified, worsening
the distress more (1, 2).

In the present investigation, carbapenem resistance
in A. baumannii was mediated by acquired CHDLs
(bla\textsubscript{OXA-23-like}, bla\textsubscript{OXA-24/40-like}, bla\textsubscript{OXA-58-like}) and less
frequently by MBLs carbapenemase. The most dis-
seminated CHDLs was bla\textsubscript{OXA-23-like} in A. baumannii
clinical isolates as reported with hospital outbreaks
(2, 5). In our study, bla\textsubscript{OXA-23-like} gene was the most
common (82.1%) oxacillinase gene detected among
CRAB isolates. This is in agreement with similar
studies conducted earlier in Iran whereby its prev-
ance varied from 83.7% to 88.7% (15, 19, 28, 30).
The prevalence of bla\textsubscript{OXA-23-like} among CRAB isolates
have been reported to vary from 44.2% to 100% in
publications from Saudi Arabia (22), Turkey (23),
Poland (24), Kuwait (26) and Algeria (31). The oth-
er prevalent oxacillinase in the current study was
bla\textsubscript{OXA-24/40-like} observed in 36.6% A. baumannii
isolates. The prevalence of this gene in Iranian studies
varied from 1.6% to 12.2% (15, 19, 28) while, a much
higher prevalence rate is evidenced in studies con-
ducted in various other parts of the world ranging
from 7.5% to 57.6% (24, 27, 31).

We found 25.8% A. baumannii isolates to carry both
bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-24/40-like} genes. Coexis-
tence of bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-24/40-like} genes is a
phenomenon which have been reported earlier from
Iran (25). In the current study, none of the CRAB
isolates were positive for bla\textsubscript{OXA-58-like} gene. This re-
result is in agreement with similar studies performed
on the prevalence of bla\textsubscript{OXA-51-like} gene among CRAB
isolates (15, 23, 28, 31). Contrary to this, presence of
bla\textsubscript{OXA-58-like} gene in CRAB isolates has been reported
from Saudi Arabia (22) and Spain (27). In our study,
all A. baumannii isolates possessed bla\textsubscript{OXA-51-like} gene.
This finding further support those of other studies
demonstrating that detection of bla\textsubscript{OXA-51-like} gene can
be used as a complementary tool to identify the or-
ganism at the species level, confirmed by additional
methods (15, 19, 20). Interestingly, similar to other
research findings (24, 25, 27), 4.4% CRAB isolates
in the present investigation were positive only for
bla\textsubscript{OXA-51-like} while, being negative for any other
bla\textsubscript{OXA-like} genes. Co-occurrence of carbapenemase
encoding genes in A. baumannii that has been
demonstrated could be linked to multiple clones har-
boring different carbapenemase encoding genes in
the same sample which may be due to multiple in-
fecions in the same individual or inter-strain horizontal
dissemination (32).

Carbapenem resistance in A. baumannii may be
associated with other mechanisms of resistance such
as modification of penicillin binding proteins, loss of
porins and decreased permeability or over expres-
sion of efflux pump (2, 4, 5). The over-expression
of CHDL encoding genes are driven mostly by pro-
motors provided by their upstream IS elements. This
element is one of the means by which A. baumannii
acquires a high level of resistance to carbapenems.
In addition to IS role as mobile promoter, they are in-
volved in mobilization of resistance genes conferring
them a high potential of diffusion (1, 8). PCR am-
plification for ISA\textsubscript{bal} in the present research study
detected this element in 95.5% of CRAB isolates.
Presence of ISA\textsubscript{bal} in CRAB isolates has been re-
ported in other related studies (19, 27, 29, 31). ISA\textsubscript{bal}
upstream of bla\textsubscript{OXA-23-like} gene was found in 69.5% of
bla\textsubscript{OXA-23-like} producing A. baumannii isolates that is
somewhat in agreement with previous studies con-
ducted in North West and North East of Iran (19, 28,
29). This finding indicate that ISA\textsubscript{bal} is associated
with bla\textsubscript{OXA-23-like} gene in most cases, may be in-
volved in overexpression of this gene, and increases
the probabilities of resistance. Association of ISA-
bal with bla\textsubscript{OXA-51-like} genes was not witnessed in our
study even though this association has been reported
in some other related studies (22, 28, 30, 31).

In agreement with other research studies (23, 27,
31), the presence of MBLs in inciting the carbapenem resistance in A. baumannii was not appreciable in comparison to blaOXA-143 genes. In the present study by using the MBL E-test strips, 33.9% (n=38) CRAB isolates were identified as MBL producers. Nevertheless, among 38 MBL producer isolates, MBL genes were confirmed only in 31.5% (n=12), while 78.5% stains were negative for any of these genes. It seems that the results of MBL detection by MBL E-test strips displayed false positivity probably due to the bactericidal activity of EDTA, which may result in increased inhibitory zone. Such phenotypic constraint necessitates implementation of molecular tests for confirmation (15).

The sensitivity and specificity of MBL phenotypic methods indicated that phenotypic methods had 100% sensitivity and 74% specificity with 31% positive predictive value but 100% negative predictive value in this study. Though for laboratories lacking molecular detection method facilities, phenotypic methods may serve an advantage however our results with high number of false positivity associated with low positive predictive value does not approve them for the detection of MBL producer strain. On the other hand, A. baumannii isolates that were phenotypically MBL producer but lacking MBL genes have been reported in other research studies (15, 21, 23, 27).

Detection of sequence groups indicated that more than 50% of the isolates in this study belonged to EC II. These strains were mainly recovered from endotracheal aspirate of patients admitted to various ICUs. EC II strains have been reported in various studies conducted earlier in Iran (30) and their distribution in various parts of the world varies from 50% to 61% (33, 34).

CONCLUSION

Waning trend in antibiotic susceptibility in ICU is of a great concern in this study. The study observed high prevalence of CRAB harboring blaOXA-23-43 and blaOXA-24-43 genes. Finding almost half of the strains belonging to EC II suggest endemicity of oxacillinase producing CRAB strains. Simultaneous presence of OXA encoding genes is a pragmatic situation that requires compliance with the rules of implementation of treatment strategies, and careful monitoring of antibiotic resistance.

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REFERENCES

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-582.
2. Pourel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-836.
3. Walsh TR, Toleman MA, Pourel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev 2005;18:306-325.
4. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of Acinetobacter baumannii: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol 2017;7:55.
5. Pourel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 2010;54:24-38.
6. Higgins PG, Lehmann M, Seifert H. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2010;35:305.
7. Higgins PG, Pérez-Llarena FJ, Zander E, Fernández A, Bou G, Seifert H. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in Acinetobacter baumannii. Antimicrob Agents Chemother 2013;57:2121-2126.
8. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett 2006;258:72-
9. Turton J, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of Acinetobacter baumannii. Clin Microbiol Infect 2007;13:807-815.

10. Hall GS (2015). Bailey & Scott’s Diagnostic Microbiology. 13th ed. American Society for Clinical Pathology. Chicago.

11. Higgins PG, Lehmann M, Wisplinghoff H, Seifert H. gyrB multiplex PCR to differentiate between Acinetobacter calcoaceticus and Acinetobacter genomic species 3. J Clin Microbiol 2010;48:4592-4594.

12. Gund VAKB, Dijkshoorn L, Burignat S, Raoult D, La Scola B. Validation of partial rpoB gene sequence analysis for the identification of clinically important and emerging Acinetobacter species. Microbiology (Reading) 2009;155:2333-2341.

13. Wayne P (2018). Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 28th ed. CLSI supplement M100s.

14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-281.

15. Shoja S, Moosavian M, Rostami S, Abbasi F, Tabatabaiefar MA, Peymani A. Characterization of oxacillinase and metallo-β-lactamases genes and molecular typing of clinical isolates of Acinetobacter baumannii in Ahvaz, south-west of Iran. Jundishapur J Microbiol 2016;9(5):e32388.

16. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006;27:351-353.

17. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119-123.

18. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol 2011;49:718-721.

19. Sohrabi N, Farajnia S, Akht MT, Nahezi MR, Naghli B, Peymani A, et al. Prevalence of OXA-type β-lactamases among Acinetobacter baumannii isolates from northwest of Iran. Microb Drug Resist 2012;18:385-389.

20. Maspi H, Mahmoodzadeh Hosseini H, Amin M, Imani Fooladi AA. High prevalence of extensively drug-resistant and metallo-beta-lactamase-producing clinical Acinetobacter baumannii in Iran. Microb Pathog 2016;98:155-159.

21. Rynga D, Shariff M, Deb M. Phenotypic and molecular characterization of clinical isolates of Acinetobacter baumannii isolated from Delhi, India. Ann Clin Microbiol Antimicrob 2015;14:40.

22. Elabed FM, Al-Ayed MSZ, Asaad AM, Alsaiei SA, Qureshi MA, Musa HA. Molecular characterization of oxacillinases among carbapenem-resistant Acinetobacter baumannii nosocomial isolates in a Saudi hospital. J Infect Public Health 2015;8:242-247.

23. Aksoy MD, Çavuşuğ Ş, Tuğrul HM. Investigation of metallo β-lactamases and oxacillinases in carbapenem resistant Acinetobacter baumannii strains isolated from inpatients. Balkan Med J 2015;32:79-83.

24. Nowak P, Puchowska P, Budak A. Distribution of blaoxa genes among carbapenem-resistant Acinetobacter baumannii nosocomial strains in Poland. New Microbiol 2012;35:317-325.

25. Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Saleghifard N, Aligholi M, et al. Antimicrobial susceptibility patterns and distribution of blaoxa genes among Acinetobacter spp. isolated from patients at Tehran hospitals. Iran J Infect Dis 2008;6:274-278.

26. Al-Sweih NA, Al-Hubail M, Rotimi VO. Three distinct clones of carbapenem-resistant Acinetobacter baumannii with high diversity of carbapenemases isolated from patients in two hospitals in Kuwait. J Infect Public Health 2012;5:102-108.

27. Villalón P, Valdezate S, Medina-Pascual MJ, Carrasco G, Vinдел A, Saez-Nieto JA. Epidemiology of the Acinetobacter-derived cephalosporinase, carbapenem-hydrolysing oxacillinase and metallo-β-lactamase genes, and of common insertion sequences, in epidemic clones of Acinetobacter baumannii from Spain. J Antimicrob Chemother 2013;68:550-553.

28. Savari M, Ekrami A, Shoja S, Bahador A. Plasmid borne Carbapenem-Hydrolyzing Class D β-Lactamases (CHDLs) and AdeABC efflux pump conferring carbapenem-tigecycline resistance among Acinetobacter baumannii isolates harboring TnAbaras. Microb Pathog 2017;104:310-317.

29. Sarhaddi N, Soleimanspou S, Farsiani H, Mosavat A, Dolatabadi S, Salimizand H, et al. Elevated prevalence of multidrug-resistant Acinetobacter baumannii with extensive genetic diversity in the largest burn centre of northeast Iran. J Glob Antimicrob Resist 2017;8:60-66.

30. Farshadzadeh Z, Hashemi FB, Rahimi S, Pourakbari B, Esmaeili D, Haghighi MA, et al. Wide distribution of carbapenem resistant Acinetobacter baumannii in burns patients in Iran. Front Microbiol 2015;6:1146.

31. Khorsi K, Messay Y, Hamidi M, Ammar H, Bakour R. High prevalence of multidrug-resistance in Acinetobacter baumannii and dissemination of carbapenemase-encoding genes blaoxa23-34, blaoxa24-44 and blaoxam1 in Algiers hospitals. Asian Pac J Trop Med 2015;8:438-446.
32. Hadjad JL, Bakour S, Rolain JM. Co-occurrence of carbapenemase encoding genes in Acinetobacter baumanii, a dream or reality? BMC Microbiol 2018;18:107.

33. Nemec A, Kržová L, Maixnerova M, Diancourt L, van der Reijden TJ, Brisse S, et al. Emergence of carbapenem resistance in Acinetobacter baumanii in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. J Antimicrob Chemother 2008;62:484–489.

34. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant Acinetobacter baumanii. J Antimicrob Chemother 2010;65:233-238.