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ORIGINAL ARTICLE

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 bla_{MBL}, $bla_{OXA\text{-}like}$, IS*Aba1* 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 $bla_{OXA\text{-}51\text{-}like}$/$bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$/$bla_{OXA\text{-}24/40\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$/$bla_{OXA\text{-}23\text{-}like}$/$bla_{OXA\text{-}24/40\text{-}like}$ was 82.1%, 36.6% and 25.8% respectively. Blending of oxacillinase and MBL genes was evident in eight $bla_{OXA\text{-}23\text{-}like}$ positive and 7 $bla_{OXA\text{-}24\text{-}like}$ positive isolates thereby depicting synchronous etiology of carbapenem resistance. Amongst CRAB isolates, 97.3% contained IS*Aba1* element and 50.9% belonged to the European clone II.
Conclusion: Synchronicity among $bla_{OXA\text{-}like}$ with bla_{MBL} and IS*Aba1* 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

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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 oxacillinases (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_{IMP}, bla_{SIM}, bla_{VIM}, bla_{GIM}, bla_{SPM} and bla_{NDM} 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 $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24/40\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}143\text{-}like}$ and $bla_{OXA\text{-}235\text{-}like}$ that are consistently associated with resistance or at least with reduced susceptibility of *A. baumannii* towards carbapenems (5-7). Although $bla_{OXA\text{-}like}$ weakly hydrolyze carbapenems, but can confer high resistance when overexpressed because of association with mobile genetic elements (ISs). 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 $bla_{OXA\text{-}51\text{-}like}$ gene, which encodes for a weak carbapenemase production. Presence of an upstream IS*Aba1* gene enhances the level of expression of this carbapenemase and has been reported as the promoter for $bla_{OXA\text{-}51\text{-}like}$ and probably, for $bla_{OXA\text{-}23\text{-}like}$ carbapenemase genes (1, 2, 8).

Many genomic fingerprinting methods utilized to determine epidemiologic links and molecular relatedness of the isolates include Repetitive Extragenic Palindromic-Polymerase 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

further analysis.

This study was approved by Ethical Committee of Tabriz University of Medical Sciences, [IR.TBZ-MEDE.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 $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24/40-like}}$ and $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-143-like}}$, $bla_{\text{OXA-235-like}}$ using primers described elsewhere (Supplementary Table). Presence of MBL genes was performed by two multiplex PCRs including bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{GIM}, bla_{SPM}, bla_{NDM} using specific primers as previously described (6, 7, 16) and (17, 18) respectively (Supplementary Table). Insertion sequence was detected in *A. baumannii* isolates using IS*Aba1* specific forward and reverse primers (IS*Aba1*-F/ISA*ba1*-R) as described previously (8) (Supplementary Table 1). For detection of IS*Aba1* linkage PCR was performed using the IS*Aba1* forward primer in combination with $bla_{\text{OXA-23-like}}$ or $bla_{\text{OXA-51-like}}$ 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 $bla_{\text{OXA-23-like}}$, and $bla_{\text{OXA-40-like}}$ 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 $bla_{\text{OXA-51-like}}$ 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)	CACGCCGTAAGAGTGCATTA	294 bp	11
gyrB- (Sp4-R)	AACGGAGCTTGTCAGGGTTA		
gyrB- (Sp2-F)	GTTCCTGATCCGAAATTCTCG	490 bp	
rpoB-F	TAYCGYAAAGAYTTGAAAGAAG		
rpoB-R	CMACACCYTTGTTMCCRTGA	350 bp	12
$bla_{\text{OXA-23-like}}$ -F	GATCGGATTGGAGAACCAGA		
$bla_{\text{OXA-23-like}}$ -R	ATTTCTGACCGCATTTCCAT		
$bla_{\text{OXA-24/40-like}}$ -F	GGTTAGTTGGCCCCCTTAAA	501 bp	16
$bla_{\text{OXA-24/40-like}}$ -R	AGTTGAGCGAAAAGGGGATT		
$bla_{\text{OXA-51-like}}$ -F	TAATGCTTTGATCGGCCTTG	246 bp	16
$bla_{\text{OXA-51-like}}$ -R	TGGATTGCACTTCATCTTGG		
$bla_{\text{OXA-58-like}}$ -F	AAGTATTGGGGCTTGTGCTG	353 bp	16
$bla_{\text{OXA-58-like}}$ -R	CCCCTCTGCGCTCTACATAC		
$bla_{\text{OXA-143-like}}$ -F	TTCTGTCAGTGCATGCTCATC	599 bp	16
$bla_{\text{OXA-143-like}}$ -R	CAGGCATTCCTTGCTTCATT		
$bla_{\text{OXA-235-like}}$ -F	TTGTTGCCTTTACTTAGTTGC	149 bp	6
$bla_{\text{OXA-235-like}}$ -R	CAAAATTTTAAGACGGATCG		
IS*Aba1*-F	CACGAATGCAGAAGTTG	768 bp	7
IS*Aba1*-R	CGACGAATACTATGACAC	549 bp	8
bla_{IMP}-F	GGAATAGAGTGGCTTAAYTCTC	188 bp	17
bla_{IMP}-R	CCAAACYACTASGTTATCT		
bla_{VIM}-F	GATGGTGTTTGGTCGCATA	390 bp	17
bla_{VIM}-R	CGAATGCGCAGCACCAG		
bla_{SIM}-F	TCGACACACCTTGGTCTGAA	570 bp	17
bla_{SIM}-R	AACTTCCAACTTTGCCATGC		
bla_{GIM}-F	TCGACACACCTTGGTCTGAA	477 bp	17
bla_{GIM}-R	AACTTCCAACTTTGCCATGC		
bla_{SPM}-F	AAAATCTGGGTACGCAAACG	271 bp	17
bla_{SPM}-R	ACATTATCCGCTGGAACAGG		
bla_{NDM}-F	GGTTTGGCGATCTGGTTTTC	621 bp	18
bla_{NDM}-R	CGGAATGGCTCATCACGATC		

Values (PPV) and Negative Predictive Values (NPV) of phenotypic test was calculated for carbapenem resistant isolates against bla_{MBL} 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, ceftazidime, ceftriaxone, imipenem, meropenem, doripenem, ciprofloxacin, levofloxacin, piperacillin/tazobactam and co-trimoxazole while, moderate susceptibility was noticed towards ampicillin-sulbactam (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 bla_{NDM} 6.2% (n=7), followed by bla_{IMP} 4.4% (n=5) while, other tested MBL genes (bla_{VIM}, bla_{SIM}, bla_{GIM} and bla_{SPM}) were not detected in any isolate.

Prevalence of the $bla_{OXA\text{-}like}$ encoding genes. All CRAB isolates carried the naturally occurring intrinsic $bla_{OXA\text{-}51\text{-}like}$ gene, 82.1% (n=92) isolates were positive for $bla_{OXA\text{-}23\text{-}like}$ and 36.6% (n=41) harbored $bla_{OXA\text{-}24/40\text{-}like}$ genes. Coexistence of three different $bla_{OXA\text{-}like}$ genes ($bla_{OXA\text{-}23\text{-}like}$ /$bla_{OXA\text{-}51\text{-}like}$/ $bla_{OXA\text{-}24/40\text{-}like}$) was a prominent feature in 25.8% (n=29) isolates. Combination of different $bla_{OXA\text{-}like}$ and bla_{MBL} genes ($bla_{OXA\text{-}23\text{-}like}$ /bla_{NDM}/bla_{IMP}) and ($bla_{OXA\text{-}24/40\text{-}like}$ /bla_{NDM}/bla_{IMP}) was detected in 7.1% (n=8) and 6.2% (n=7) isolates, respectively (Figs. 1 and 2). Furthermore, 4.4% (n=5) isolates were positive for only $bla_{OXA\text{-}51\text{-}like}$ gene, lacking other $bla_{OXA\text{-}like}$ carbapenemase genes. None of the *A. baumannii* isolate was positive for $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}143\text{-}like}$ and $bla_{OXA\text{-}235\text{-}like}$ genes (Fig. 1).

Prevalence of IS*Aba1* upstream of $bla_{OXA\text{-}23\text{-}like}$ gene. IS*Aba1* element was found in 95.5% (n=107) *A. baumannii* isolates. In sixty-four (69.5%) isolates with $bla_{OXA\text{-}23\text{-}like}$, IS*Aba1* lay upstream of $bla_{OXA\text{-}23\text{-}like}$ however, IS*Aba1* was not detected upstream of $bla_{OXA\text{-}51\text{-}like}$ 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: Ceftazidime, CTX: Cefotaxime, CRO: Ceftriaxone, CIP: Ciprofloxacin, LEV: Levofloxacin, SXT: Co-trimoxazole, PTZ: Piperacillin/tazobactam, GM: Gentamicin, AK: Amikacin, TOB: Tobramycin, AMS: Ampicillin/sulbactam

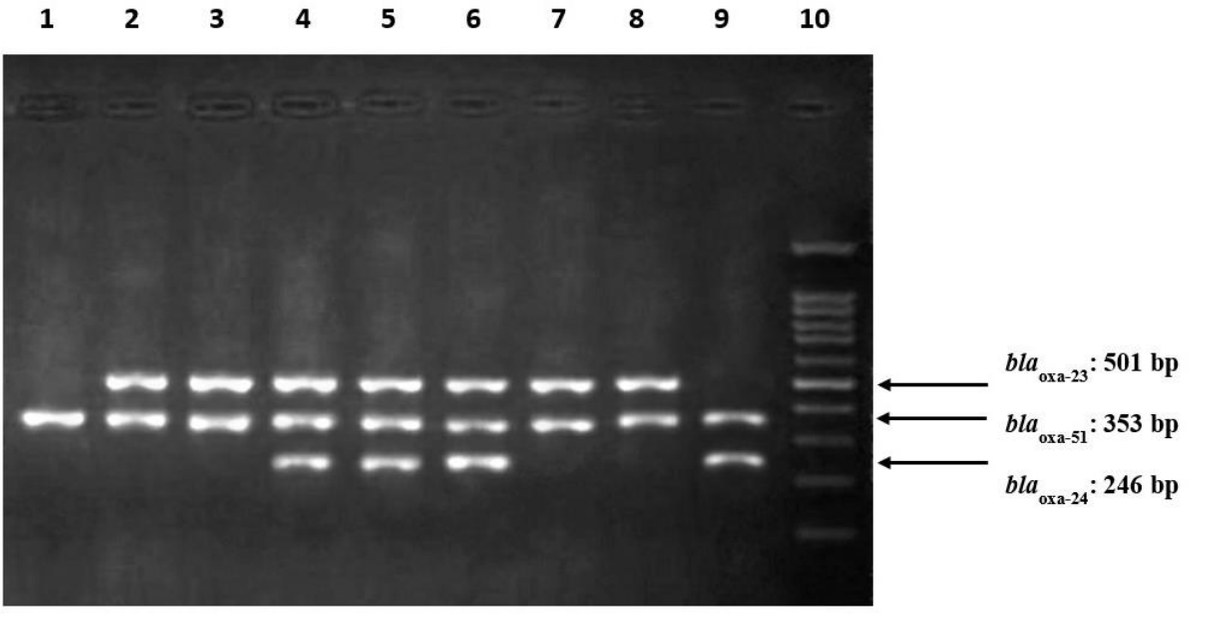

Fig. 1. PCR analysis depicting $bla_{\text{OXA-like}}$ encoding genes

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

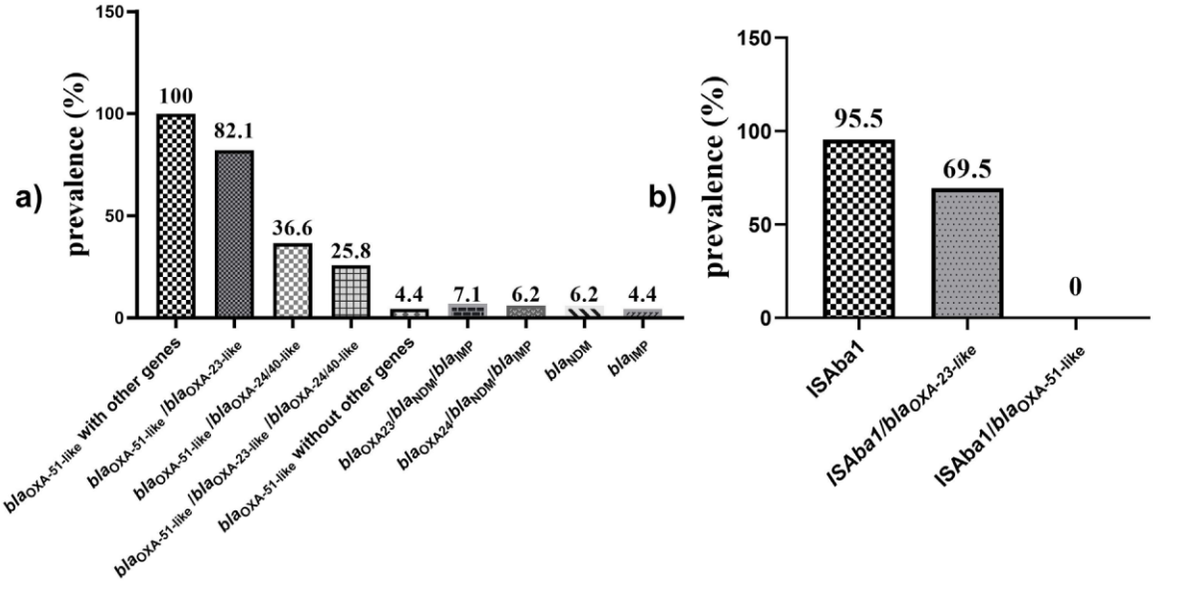

Fig. 2. Prevalence of OXA, MBL and IS*Ab1* genes in *A. baumannii* clinical isolates

a) Prevalence of oxacillinase and metallo-β-lactamase genes
b) Prevalence of IS*Aba1* and IS*Aba1* upstream of oxacillinase genes

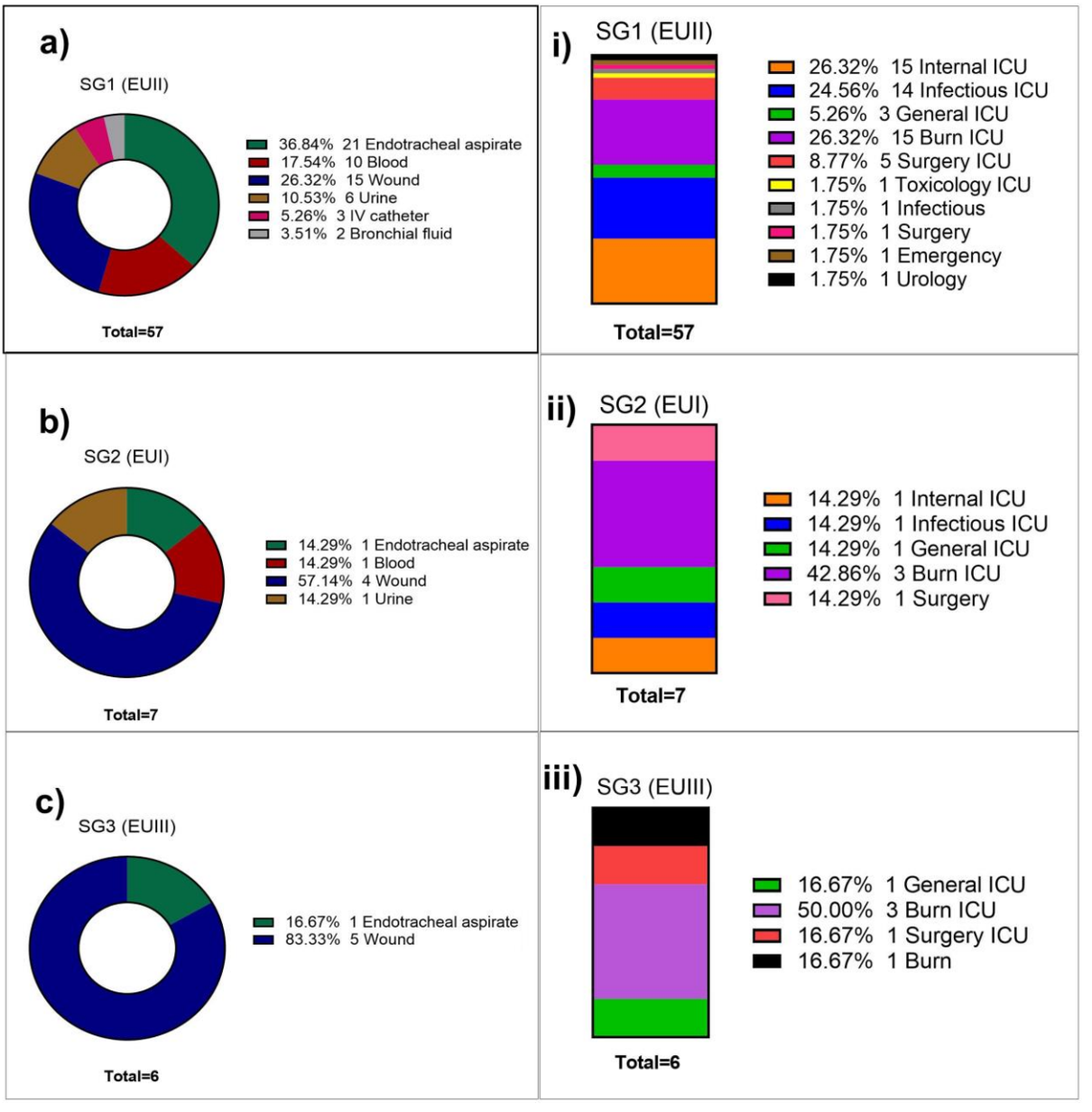

Fig. 3. Source of *A. baumannii* clinical isolates in three sequence groups

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
i) Distribution of SG1 (EUII) in various hospital wards
ii) Distribution of SG2 (EUI) in various hospital wards
iii) Distribution of SG3 (EUIII) in various hospital wards

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

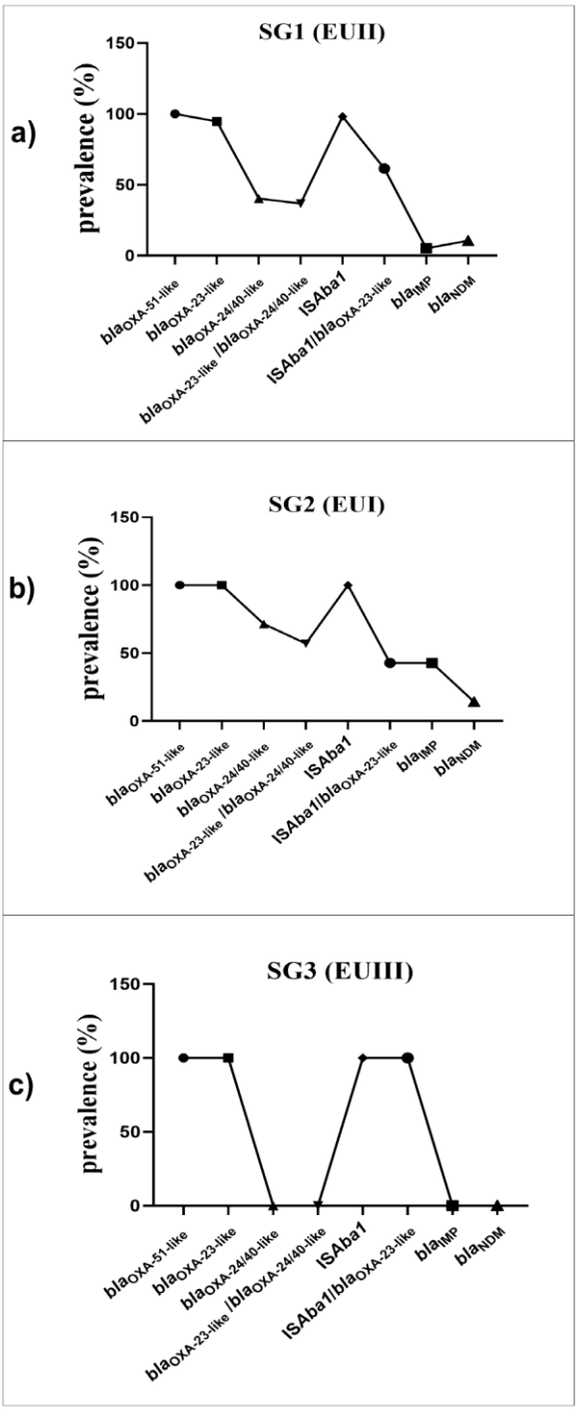

Fig. 4. Prevalence of carbapenemase genes in three sequence groups

a) Prevalence of OXA, MBL genes and IS*Aba1* in SG1 (EUII)
b) Prevalence of OXA, MBL genes and IS*Aba1* in SG2 (EUI)
c) Prevalence of OXA, MBL genes and IS*Aba1* in SG3 (EUIII)

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

as these antibiotics were the last resort of therapeutic 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 efficacy against *A. baumannii* with susceptibility rate of 100%. Though this finding is consistent with most studies conducted earlier in Iran (15, 28, 30) nevertheless, 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 infections and as evidenced there is tendency that with overuse its resistance rate may increase. Indeed, *A. baumannii* isolates resistant to carbapenem and colistin simultaneously have been identified, worsening the distress more (1, 2).

In the present investigation, carbapenem resistance in *A. baumannii* was mediated by acquired CHDLs ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24/40-like}}$, $bla_{\text{OXA-58-like}}$) and less frequently by MBLs carbapenemase. The most disseminated CHDLs was $bla_{\text{OXA-23-like}}$ in *A. baumannii* clinical isolates as reported with hospital outbreaks (2, 5). In our study, $bla_{\text{OXA-23-like}}$ 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 prevalence varied from 83.7% to 88.7% (15, 19, 28, 30). The prevalence of $bla_{\text{OXA-23-like}}$ 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 other prevalent oxacillinase in the current study was $bla_{\text{OXA-24/40-like}}$ 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 conducted 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 $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24/40-like}}$ genes. Coexistence of $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24/40-like}}$ genes is a phenomenon which have been reported earlier from Iran (25). In the current study, none of the CRAB isolates were positive for $bla_{\text{OXA-58-like}}$ gene. This result is in agreement with similar studies performed on the prevalence of $bla_{\text{OXA-58-like}}$ gene among CRAB isolates (15, 23, 28, 31). Contrary to this, presence of $bla_{\text{OXA-58-like}}$ gene in CRAB isolates has been reported from Saudi Arabia (22) and Spain (27). In our study, all *A. baumannii* isolates possessed $bla_{\text{OXA-51-like}}$ gene. This finding further support those of other studies demonstrating that detection of $bla_{\text{OXA-51-like}}$ gene can be used as a complementary tool to identify the organism 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 $bla_{\text{OXA-51-like}}$ while, being negative for any other $bla_{\text{OXA-like}}$ genes. Co-occurrence of carbapenemase encoding genes in *A. baumannii* that has been demonstrated could be linked to multiple clones harboring different carbapenemase encoding genes in the same sample which may be due to multiple infections 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 expression of efflux pump (2, 4, 5). The over-expression of CHDL encoding genes are driven mostly by promoters 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 involved in mobilization of resistance genes conferring them a high potential of diffusion (1, 8). PCR amplification for IS*Aba1* in the present research study detected this element in 95.5% of CRAB isolates. Presence of IS*Aba1* in CRAB isolates has been reported in other related studies (19, 27, 29, 31). IS*Aba1* upstream of $bla_{\text{OXA-23-like}}$ gene was found in 69.5% of $bla_{\text{OXA-23-like}}$ producing *A. baumannii* isolates that is somewhat in agreement with previous studies conducted in North West and North East of Iran (19, 28, 29). This finding indicate that IS*Aba1* is associated with *blaOXA-23-like* gene in most cases, may be involved in overexpression of this gene, and increases the probabilities of resistance. Association of IS*Aba1* with $bla_{\text{OXA-51-like}}$ 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 $bla_{\text{OXA-like}}$ 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 $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ 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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