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ORIGINAL ARTICLE

In vitro activity of colistin against multidrug-resistant *Acinetobacter baumannii* isolates harboring $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes: A multicenter based study

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ABSTRACT

This study was aimed to evaluate occurrence of antibiotic resistance and the presence of resistance determinants among clinical isolates of *Acinetobacter baumannii*. This cross-sectional study from January to September 2018 was performed on 59 *A. baumannii* strains isolated from clinical samples in the north of Iran. Isolates were identified by standard microbiologic tests and molecular method. Antimicrobial susceptibility testing was carried out by disk diffusion and broth microdilution methods. The presence of carbapenem resistance genes was detected by PCR method. All isolates were resistant to cefepime, meropenem, imipenem and ceftazidime. The lowest resistance rate was observed against doxycycline with 33.9%. Minimum inhibitory concentration (MIC) results showed that all carbapenem-resistant *A. baumannii* (CRAB) isolates were susceptible to colistin with MIC50 and MIC90 values of 1/2 μg/mL. Among 59 CRAB, $bla_{\text{OXA-23-like}}$ was the most prevalent gene (86.4%) followed by $bla_{\text{OXA-24-like}}$ (69.5%). Meanwhile, none of the clinical isolates harbored $bla_{\text{OXA-58-like}}$ gene. We found a high prevalence of CRAB strains harboring OXA-type carbapenemases in the north of Iran. Our results suggests that the presence of OXA-type genes was not directly correlated with the increase of imipenem MIC level, but can be clinically important as they contribute to the selection of CRAB strains.

KEYWORDS

Acinetobacter baumannii, carbapenem resistance, OXA-type carbapenemases, colistin, extensively-drug resistant

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INTRODUCTION

Acinetobacter baumannii is a major cause of hospital-acquired infections, particularly in intensive care units (ICUs) [1, 2]. This bacterium is associated with a wide range of infections including skin and soft tissues, burned wound, urinary tract, gastrointestinal, and respiratory tracts infections [2, 3]. Nowadays, the increasing prevalence of infections caused by multiple-(MDR) and extensively-drug resistant (XDR) *A. baumannii* is an excessive public health concern [4, 5]. Routinely, carbapenems were highly efficacious antibiotics for treatment of severe infections caused by MDR strains [6]. Unfortunately, a global rise in trend of

carbapenem-resistant *A. baumannii* (CRAB) strains has been reported during the last decade [4, 7]. The production of class D β-lactamase, also known as oxacillinases (OXA) is the major mechanism of resistance to carbapenems in *A. baumannii* [8]. This bacterium predominantly expresses OXA-type enzymes such as the intrinsic OXA-51 enzyme, and the acquired OXA-23, OXA-24 (OXA-40), OXA-58 [8, 9]. Different plasmids can harbor the OXA-type enzymes genes which could result in a rapid dissemination among *A. baumannii* strains and treatment difficulties [9].

Limited therapeutic options to treat infections caused by these drug-resistant strains request development of new drugs or recruiting the old ones [10]. Colistin (also known as polymyxin E) is a multicomponent polypeptide antibiotic and relatively old polymyxin that achieved its antimicrobial effect by acting directly on bacterial cell membrane. [11] Currently, colistin is a reliable option for treatment of infections caused by MDR Gram-negative bacteria [10, 12].

Due to the lack of data from our region and also high horizontal gene transferring capability of *A. baumannii*, it is necessary to estimate the burden of carbapenem-resistant strains in hospitals. Therefore, this study was aimed to evaluate the occurrence of antibiotic resistance and the presence of resistance determinants among *A. baumannii* strains isolated from hospitalized patients. Findings of the present study can be used for the improvement of available infection control policies and also to contribute to the international data on the antimicrobial stewardship programs.

MATERIALS AND METHODS

Study design and bacterial identification

In a cross-sectional study during nine month period from January to September 2018, clinical samples were collected from hospitalized patients with ventilator-associated pneumonia (VAP), burned wounds and bloodstream infections (BSIs) in ICUs of three teaching hospitals in the north of Iran. This study was in accordance with the declaration of Helsinki and study design was approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1397.130). We also received informed consent from all patients. Samples were cultured on MacConkey agar plates and incubated aerobically at 37 °C for 24 h. Then all non-fermentative Gram negative rods were identified as *A. baumannii* by standard microbiological tests including oxidase negative, catalase positive, nonmotile and growth at 41 °C. Additionally, all presumptive isolates were confirmed by amplification of $bla_{\text{OXA-51-like}}$ gene as it is exclusive to *A. baumannii* species [13].

Antimicrobial susceptibility testing

Antibiotic susceptibility test was performed by disk-diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI-M100-S28) to the following antimicrobial agents, ampicillin/sulbactam (10/10 µg), meropenem (10 µg), gentamicin (10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), cefepime (30 µg), ceftazidime (30 µg), and levofloxacin (30 µg) (Mast, UK). Results were interpreted in accordance with CLSI guidelines 2018 [14]. Briefly, each bacterial sample was suspended in sterile normal saline and reached to a standard turbidity equal to 0.5 McFarland. All prepared suspensions were cultured on Mueller–Hinton agar (Merck, Germany) plates and incubated at 37 °C for 16–18 h, and the zones of inhibition were measured and recorded. Colistin minimum inhibitory concentration (MIC) was determined by broth microdilution in accordance with the CLSI/EUCAST recommendations using colistin sulfate powder (Sigma–Aldrich, St. Louis, MO) [15]. Twofold drug dilutions ranging from 0.125 to 128 µg/mL were prepared for colistin and the test was performed using a 96-well polystyrene plate in cation-adjusted Mueller–Hinton broth (Merck, Germany). For imipenem, MIC was determined by Epsilometer (E)-test strips (Liofilchem, Italy) on Mueller–Hinton agar plates according to the manufacturer's specification. MIC results for colistin and imipenem were interpreted according to the CLSI 2018 breakpoints. MIC50 and MIC90 (MIC at which 50% and 90% of isolates were inhibited) were estimated and reported for each individual antibiotic. *Pseudomonas aeruginosa* ATCC 27853 was used as reference strains for susceptibility testing. According to Magiorakos et al. estimation, MDR was defined as non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories and extensively-drug-resistant (XDR) defined as non-susceptible to ≥ 1 agent in all but < 2 categories [16].

DNA extraction and amplification of the bla_{OXA} genes

The bacterial whole genome was extracted by boiling method as previously described [17]. Multiplex polymerase chain reaction (M-PCR) was used for detecting of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ genes [13]. Primers used for bla_{OXA} genes amplifying are shown in Table 1. M-PCR were performed in a final volume of 50 µL including 50 ng of extracted genomic DNA, 20 pM of each primer, 10 µL Master mix (Ampliqon, Denmark). Amplification conditions were done by initial denaturation at 94 °C for 3 min followed by 30 cycles of 25 s at 94 °C, 40 s at 52 °C and 50 s at 72 °C with a final extension of 5 min at 72 °C. PCR products were electrophoresed in 1.5% agarose gel containing safe stain (CinnaGen Co., Iran) and visualized under ultraviolet light.

Statistical analysis

Analysis was performed using SPSS™ software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the mean ± standard deviation (continuous variables) or percentages of the group (categorical variables). Chi-square or Fisher's exact tests were used to determine the significance of differences. A difference was considered statistically significant if the p value was less than 0.05.

Table 1. Primers sequences used in this study

Genes	Primers	Sequences (5′→3′)	Size (bp)	References
$bla_{\text{OXA-51}}$	Forward	TAATGCTTTGATCGGCCTTG	353	[15]
	Reverse	TGG ATTGCACTTCATCTTGG		
$bla_{\text{OXA-23}}$	F	GAT CGG ATT GGA GAA CCA GA	501	[15]
	R	ATT TCT GAC CGC ATT TCC AT		
$bla_{\text{OXA-24}}$	F	GGT TAG TTG GCC CCC TTA AA	246	[15]
	R	AGT TGA GCG AAA AGG GGA TT		
$bla_{\text{OXA-58}}$	F	AAG TAT TGG GGC TTG TGC TG	599	[15]
	R	CCC CTC TGC GCT CTA CAT AC		

RESULTS

Demographic data

Totally, 59 (8.4%) non-duplicated (one per patient) *A. baumannii* isolates were obtained from clinical samples. Of 59 samples, 39 (66.1%) obtained from male and 20 (33.9%) from female patients. Patients' ages were ranged from 15 to 88 years old with a mean age of 53.6 years. The majority of *A. baumannii* strains were isolated from VAP (71.2%) followed by burned wounds (20.3%) and BSIs (8.5%).

Antibiotic susceptibility pattern

The results of antibiotic susceptibility test revealed that all isolates were resistant to cefepime, meropenem, imipenem, and ceftazidime. The lowest resistance rate was observed against doxycycline with 33.9% (Table 2). MIC results showed that all clinical isolates of *A. baumannii* were susceptible to colistin with MIC50 and MIC90 of 1/2 µg/mL, while all were resistant to imipenem with MIC50 and MIC90 of >32/ >32 µg/mL. Moreover, 8.5% and 91.5% of isolates were considered as MDR and XDR, respectively.

Prevalence of OXA-type carbapenemases *genes*

M-PCR results revealed that among 59 clinical isolates of *A. baumannii*, $bla_{\text{OXA-23-like}}$ was the most prevalent gene (86.4%) followed by $bla_{\text{OXA-24-like}}$ (69.5%). Besides, 59.3% of isolates were carrying both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes simultaneously. Meanwhile, none of the clinical isolates harbored $bla_{\text{OXA-58-like}}$ gene. There was no significant association between the presence of OXA-type genes and higher antibiotic resistance rate (Data not shown).

The cumulative percentage of OXA-type harboring isolates inhibited at each imipenem and colistin MIC value are shown in Table 3 and Table 4, respectively. Comparing with OXA-type negative isolates, we found that OXA-type positive isolates were inhibited more likely at an imipenem MIC ≤24 µg/mL, while they were inhibited at a higher colistin MIC value.

DISCUSSION

In accordance with recent reports from Iran [5, 7], the prevalence of carbapenem (imipenem and meropenem) resistance in our region was high (100%). Several factors including misuse and overuse of antibiotics, poor infection control practices, poor antimicrobial stewardship programs, poor hand hygiene practices, and neighboring with high CRAB burden countries addressed to the recent emergence of CRAB in Iran. [7, 18] It has been suggested that the main mechanism of carbapenem resistance *in A. baumannii* is the presence of the OXA-type carbapenemases [8]. But to investigate the clinical impact of these genes on CRAB isolates we evaluate the MIC of imipenem and colistin toward OXA-type positive isolates.

Our findings revealed that 86.4% and 69.5% of CRAB isolates harbored $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes, respectively, but $bla_{\text{OXA-58-like}}$ had no role in resistance to carbapenem. Despite the regional variations in Iran, our results are closest with the recent meta-analysis reports of OXA-23 (73.7%; 95% CI 66.5–79.8) and OXA-58 (6.2%; 95%

Table 2. Antibiotic resistance pattern of tested isolates

Class	Antibiotic	Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)
ß-Lactam combination agents	Ampicillin/sulbactam	0	4 (6.8)	55 (93.2)
Cephems	Ceftazidime	0	0	59 (100)
	Cefepime	0	0	59 (100)
Aminoglycosides	Gentamicin	1 (1.7)	0	58 (98.3)
Tetracyclines	Doxycycline	39 (66.1)	0	20 (33.9)
Fluoroquinolones	Ciprofloxacin	1 (1.7)	0	58 (98.3)
	Levofloxacin	1 (1.7)	1 (1.7)	57 (96.6)
Sulfonamide	Co-trimoxazole	3 (5.1)	7 (11.9)	49 (83)
Carbapenems	Meropenem	0	0	59 (100)
	Imipenem	0	0	59 (100)
Polymyxins	Colistin	59 (100)	0	0

Table 3. The cumulative percentage of isolates inhibited at each imipenem MIC value

Variable (no. of isolates)	Cumulative number (%) of isolates inhibited at MIC value (**μg/mL**)				
	8	12	16	24	>32
$bla_{\text{OXA-23-like}}$ negative (8)	0	1 (12.5)	0 (12.5)	0 (12.5)	7 (100)
$bla_{\text{OXA-23-like}}$ positive (51)	1 (2)	2 (5.9)	7 (19.6)	1 (21.6)	40 (100)
$bla_{\text{OXA-24-like}}$ negative (18)	0	1 (5.6)	1 (11.1)	0 (11.1)	16 (100)
$bla_{\text{OXA-24-like}}$ positive (41)	1 (2.4)	2 (7.3)	6 (22)	1 (24.4)	31 (100)
Both genes negative (24)	0	1 (4.2)	1 (8.3)	0 (8.3)	22 (100)
Both genes positive (35)	1 (2.9)	2 (8.6)	6 (25.7)	1 (28.6)	25 (100)

Table 4. The cumulative percentage of isolates inhibited at each colistin MIC value

Variable (no. of isolates)	Cumulative number (%) of isolates inhibited at MIC value (**μg/mL**)				
	0.125	0.25	0.5	1	2
$bla_{\text{OXA-23-like}}$ negative (8)	0	0	3 (37.5)	3 (75)	2 (100)
$bla_{\text{OXA-23-like}}$ positive (51)	4 (7.8)	1 (9.8)	14 (37.3)	7 (51)	25 (100)
$bla_{\text{OXA-24-like}}$ negative (18)	2 (11.1)	1 (16.7)	5 (44.4)	3 (61.1)	7 (100)
$bla_{\text{OXA-24-like}}$ positive (41)	2 (4.9)	0 (4.9)	12 (34.1)	7 (51.2)	20 (100)
Both genes negative (24)	2 (8.3)	1 (12.5)	8 (45.5)	6 (70.8)	7 (100)
Both genes positive (35)	2 (5.7)	0 (5.7)	9 (31.4)	4 (42.9)	20 (100)

CI 3.1–11.9), while it was higher than OXA-24 (21.9%; 95% CI 15.2–30.4) report [7]. Similar to our findings, Zowawi et al. introduced OXA-23 and OXA-24 as the most circulating carbapenemases in CRAB strains isolated from Gulf Cooperation Council [19]. Also, in a study from Brazil, 87% of *A. baumannii* isolates presented OXA-23 and 13% OXA-24, but OXA-58 was not detected [20]. Ahmed et al. showed OXA-23 (67%) as the most prevalent carbapenemases among *A. baumannii* strains isolated from Turkey and Azerbaijan followed by OXA-58 (6.2%), and OXA-24 (4.5%) [21]. Same report was cited from China, where OXA-23 (30.3%) was the most common carbapenemases followed by OXA-58 (27.2%), and OXA-40 (0.3%) [22].

In the present study, OXA-type positive isolates were inhibited at a lower imipenem concentration compared to negative isolates, suggesting that the presence of these genes is not the only mechanism of carbapenem resistance in our region. These findings may be due to following reasons including a low number of OXA-type negative isolates compare to positive ones in our study, the absence of RND type efflux systems [23, 24], and the absence of insertion sequences (such as *ISAba1*) [25, 26]. However, it has been found that the presence or multiplication of OXA-23 does not necessarily enhance carbapenem resistance [27, 28].

Carbapenem resistance is a serious obstacle to control the spread and treatment of *A. baumannii* strains. In our results, all CRAB isolates were susceptible to colistin with a MIC value ≤2 μg/mL. Polymyxins are usually drug of choice to treat infections caused by MDR Gram-negative bacteria [12]. Previously two comparable reports were available from southern region [10, 29], but our study was the first report from our region in the north of Iran. Moreover, our results are consistent with reports from CANWARD study and SENTRY program suggests that colistin remains an available option for the treatment of infection caused by CRAB since the majority of isolates were susceptible to colistin [30, 31].

CONCLUSION

In summary, we found a high prevalence of CRAB strains harboring OXA-type carbapenemases in the north of Iran. Our results suggest that the presence of OXA-type genes was not directly correlated with the increase of imipenem MIC level but can be clinically important as they contribute to the selection of CRAB strains. However, further studies are required to investigate all other possible mechanisms of carbapenem resistance.

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