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HIGH PREVALENCE OF OXA-TYPE CARBAPENEMASES AMONG *ACINETOBACTER BAUMANNII* STRAINS IN A TEACHING HOSPITAL OF TEHRAN

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Nosocomial infection caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) has created a public health concern all around the world. In this study, 100 isolates of CRAB from hospitalized patients during 2015–2016 at Imam Khomeini Hospital were investigated to determine the rates of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains using Kirby–Bauer disk diffusion method. The minimum inhibitory concentrations (MICs) of six antibiotics were determined by broth microdilution method. Multiplex polymerase chain reaction (PCR) was performed to detect $bla_{\text{OXA-51 like}}$ and $bla_{\text{OXA-58 like}}$, $bla_{\text{OXA-23 like}}$, and $bla_{\text{OXA-24 like}}$ that are encoding resistance to carbapenems. All CRAB isolates were MDR and XDR and 2% of them were pandrug-resistant (PDR), whereas colistin, polymyxin B, and tigecycline were the most effective agents. All isolates were positive for $bla_{\text{OXA-51 like}}$ by PCR. The frequency of $bla_{\text{OXA-23 like}}$ and $bla_{\text{OXA-24 like}}$ was 81% and 22%, respectively. Findings of this study showed that very few therapeutic options remained for the treatment of CRAB infections and $bla_{\text{OXA-23 like}}$ is a dominant resistance gene in CRAB at this hospital.

Keywords: CRAB, MDR, XDR, multiplex PCR, $bla_{\text{OXA-51 like}}$, $bla_{\text{OXA-58 like}}$, $bla_{\text{OXA-23 like}}$, $bla_{\text{OXA-24 like}}$

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Introduction

Acinetobacter baumannii (A. baumannii) is one of the major hospital-associated and an opportunistic pathogen that causes serious diseases in respiratory tract, bloodstream, urinary tract, at surgical site, and wound infections [1]. *Acinetobacter* spp. have intrinsic resistance to many antibiotics and disinfectants due to low permeability of outer cell membrane and expression of efflux pumps; furthermore, it can survive for long period of time on hospital inanimate surfaces and equipment [2]. The Infectious Diseases Society of America expressed that *A. baumannii* is one of the six antimicrobial-resistant pathogens that are responsible for high morbidity and mortality in patients. Multidrug-resistant (MDR) strains of *A. baumannii* (resistant to at least three different classes of antimicrobial agents, such as cephalosporins, aminoglycosides, and fluoroquinolones) emerged in the last 2 decades [1].

Carbapenems are the drug of choice for MDR strains of *A. baumannii* because of their potent bactericidal activity against them [3]. However, *A. baumannii* develops resistance to carbapenems in all over the world with different mechanisms, such as decreased permeability, altered penicillin-binding proteins or porins (33-kDa CarO protein) that are causing an influx channel for carbapenems and overexpression of efflux pumps. Carbapenem-hydrolyzing class D β-lactamases (CHDLs) and class B carbapenemase production are important carbapenem-resistant mechanisms. The CHDLs (OXA carbapenemases) of *Acinetobacter* spp. have five phylogenetic subgroups: $bla_{\text{OXA-51 like}}$, $bla_{\text{OXA-58 like}}$, $bla_{\text{OXA-23 like}}$, $bla_{\text{OXA-24/40 like}}$, and $bla_{\text{OXA-143 like}}$ [3–9]. The $bla_{\text{OXA-51 like}}$ gene is an intrinsic gene for *A. baumannii* strains, however, alone $bla_{\text{OXA-51like}}$ gene cannot represent the increase of carbapenem resistance, because insertion of ISAba1 or similar IS elements is required in *A. baumannii* to gain the carbapenem resistance [10]. Scotland was the first where $bla_{\text{OXA-23 like}}$ gene was characterized and after that the outbreak of this gene has been reported from many countries, such as Bulgaria, People's Republic of China, Brazil, Iran, Iraq, Afghanistan, and French Polynesia. In many studies, the relationship between the presence of $bla_{\text{OXA-23 like}}$ and resistance to imipenem and meropenem has been demonstrated [11].

The frequency of carbapenem-resistant Acinetobacter baumannii (CRAB) at the hospitals of the United States varies from 33% to 58% [1] and infections caused by these organisms have only limited number of therapeutic options [8]. In cases with CRAB, the physician must use more toxic agent, such as colistin, a cationic peptide antibiotic that becomes active drug in the blood [12–14]. However, clinical isolates of *A. baumannii* have acquired resistance to colistin

more recently. The US hospitals in a surveillance study reported that the rate of colistin resistance among *Acinetobacter* spp. is 5.3% [15]. Disturbance of the electrostatic interactions between positively charged colistin molecules and the negatively charged lipopolysaccharide of bacterial membrane reduces the activity of colistin [13]. This study described the frequency of CRAB during a 9-month study and investigated the susceptibility of them to other antibiotics. Furthermore, the presence of $bla_{\text{OXA-23 like}}$, $bla_{\text{OXA-24 like}}$, $bla_{\text{OXA-51 like}}$, and $bla_{\text{OXA-58 like}}$ genes was investigated with multiplex polymerase chain reaction (PCR) to determine the relationship between the presence of these genes and resistant to carbapenems.

Materials and Methods

Isolation and identification of CRAB

A. baumannii isolates were collected from blood, wound, urine, sputum, and respiratory tract at different wards of Imam Khomeini Hospital in Tehran from September 2015 to June 2016. The primary identification of *A. baumannii* isolates was carried out by biochemical tests [16]. One hundred of *A. baumannii* isolates that were resistant to imipenem and meropenem were randomly collected as CRAB, and then stored in trypticase soy broth and glycerol at −70 °C until use [17].

Antimicrobial susceptibility analysis

The susceptibility of CRAB isolates, to determine MDR and extensively drug-resistant (XDR) and pandrug-resistant (PDR) phenotypes, was performed using Kirby–Bauer disk diffusion method according to the CLSI guidelines [18, 19] to various classes of antibiotics, such as gentamicin (10 μg), amikacin (30 μg), ampicillin (10 μg), ampicillin/sulbactam (20 μg), trimethoprim/sulfamethoxazole (25 μg), piperacillin/tazobactam (100/10 μg), tetracycline (30 μg), ticarcillin (75 μg), ticarcillin/clavulanic acid (75/10 μg), tobramycin (10 μg), netilmicin (30 μg), cefepime (30 μg) cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), ofloxacin (5 μg), meropenem (10 μg), imipenem (10 μg), doripenem (10 μg), doxycycline (30 μg), minocycline (30 μg), tigecycline (10 μg), colistin (10 μg), polymyxin B (300 units) (MAST, Merseyside, UK) [19]. The CRABs that were non-susceptible to at least one agent in all but two or fewer antimicrobial categories and the isolates that were resistant to all available antibacterial agents except

polymyxin B and colistin were called XDR and PDR, respectively [17, 19, 20]. Minimum inhibitory concentrations (MICs) of imipenem, meropenem, colistin, amikacin, cefotaxime, and ampicillin/sulbactam were determined using broth microdilution method. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains [1, 18].

Multiplex PCR for evaluation of bla_{OXA} *genes*

Multiplex PCR was carried out to detect $bla_{\text{OXA-51 like}}$, $bla_{\text{OXA-23 like}}$, $bla_{\text{OXA-24 like}}$, and $bla_{\text{OXA-58 like}}$ that are encoding carbapenem-resistance genes using the primers described by Woodford et al. [8].

A. baumannii strains including NCTC 13304, NCTC 13302, NCTC 12156, and NCTC 13305 were used as positive controls for $bla_{\text{OXA-23 like}}$, $bla_{\text{OXA-24 like}}$, $bla_{\text{OXA-58 like}}$, and $bla_{\text{OXA-51 like}}$, respectively [18]. The multiplex PCR protocol was the same as described by Woodford et al. [8].

Results

During the 9-month study period starting from September 2015, altogether 3,731 clinical specimens were referred to the Imam Hospital Laboratory for bacteriology tests and were positive in culture, of these 536 yielded *A. baumannii*.

The prevalence of MDR and CRAB phenotypes among all isolates of *A. baumannii* during the study was 83% ($n = 445$) and 80% ($n = 429$), respectively. The randomly selected isolates in this study ($n = 100$) were recovered from patients at ICUs ($n = 43$), emergency ($n = 35$), surgical wards ($n = 7$), and medical ($n = 15$). The highest numbers of specimens belonged to ICUs. The $bla_{\text{OXA-51 like}}$ was observed in all 100 CRAB isolates.

As shown in Table I, tracheal aspirates ($n = 44$) were the most clinical specimens referred to the laboratory followed by blood ($n = 38$). About 58% of patients were males and 42% were females. Table II shows the antibiotic resistance pattern. Two isolates showed PDR phenotype, but all of them were MDR and XDR (100%). The most effective antimicrobial agents were colistin and polymyxin with two and five resistant strains, respectively. Tigecycline (69%), minocycline (49%), and doxycycline (40%) were also effective antibiotics. MIC_{50} and MIC_{90} values of *A. baumannii* isolates are shown in Table III. Colistin and ampicillin/sulbactam had the lowest MIC values. Imipenem and amikacin and cefotaxime and meropenem had the highest MIC values, respectively (Table III).

Table I. Distribution of *A. baumannii* isolates that is collected from different clinical specimens

Specimen	Number
Blood	38
Urine	9
Tracheal aspirates	44
Pulmonary secretion	4
Wound	1
Catheter	1

Table II. Resistance range of *A. baumannii* isolates to various antimicrobial agents

Antibiotics	Sensitive	Intermediate	Resistant
Gentamicin	1	0	99
Tobramycin	7	1	92
Amikacin	1	0	99
Netilmicin	8	2	90
Imipenem	0	0	100
Meropenem	0	0	100
Doripenem	0	0	100
Ciprofloxacin	1	0	99
Levofloxacin	19	22	59
Ofloxacin	2	3	95
Piperacillin/tazobactam	0	1	99
Ticarcillin	0	0	100
Ticarcillin/clavulanic acid	0	0	100
Cefotaxime	1	0	99
Ceftriaxone	0	0	100
Ceftazidime	2	1	97
Cefepime	0	0	100
Trimethoprim/ sulfamethoxazole	2	1	97
Ampicillin	0	1	99
Ampicillin/sulbactam	2	6	92
Colistin	98	0	2
Polymyxin B	95	–	5
Tetracycline	18	11	71
Doxycycline	40	2	58
Minocycline	49	9	42
Tigecycline	69	29	2

The frequency of $bla_{\text{OXA-23 like}}$ and $bla_{\text{OXA-24 like}}$ was 81% and 22%, respectively. The $bla_{\text{OXA-58 like}}$ gene was not found at CRAB isolates. The coexistence of $bla_{\text{OXA-23 like}}$ *and* $bla_{\text{OXA-24 like}}$ was 19% (Figure 1).

Table III. The MIC_{50} and MIC_{90} values of *A. baumannii* isolates

Antibiotics	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	Mode (μg/ml)
Imipenem	1,280	1,280	0.625–1,280	1,280
Meropenem	80	320	0.625–1,280	40
Cefotaxime	640	1,280	0.625–1,280	640
Amikacin	1,280	1,280	0.625–1,280	1,280
Colistin	0.3	0.83	0.15625–1,280	0.16
Ampicillin/sulbactam	80	160	0.625–1,280	80

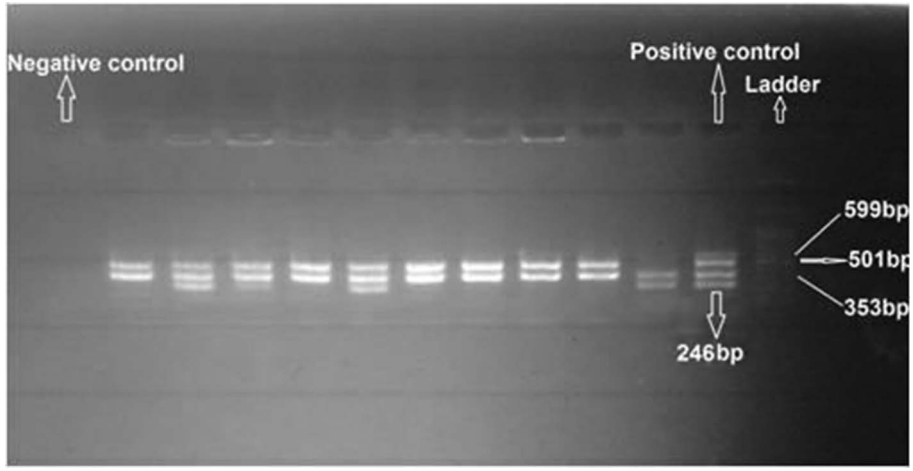

Figure 1. Multiplex PCR of *A. baumannii* isolates: $bla_{\text{OXA-51like}}$ (353 pb), $bla_{\text{OXA-23 like}}$ (501 bp), $bla_{\text{OXA-24 like}}$ (246 bp), and $bla_{\text{OXA-23 like}}$ (599 bp)

Discussion

The results of bacteriology showed high prevalence of infections with MDR and XDR strains of *A. baumannii* at different wards of Imam Khomeini Hospital. The mortality rates of nosocomial infections with *A. baumannii* at the US hospitals have been reported between 19% and 54% [20]. In this study, 536 isolates of *A. baumannii* isolates were cultured and 90% of them were resistant to carbapenems. Among the CRAB isolates, 100 were further investigated. The identification of isolates was confirmed by PCR as all of them were positive for $bla_{\text{OXA-51 like}}$. Turton et al. [10] in 2006 demonstrated that detection of $bla_{\text{OXA-51 like}}$ gene is a reliable method for identification of *A. Baumannii*. Similar findings were also reported from Iran [20].

The majority of isolates recovered in this study were cultured from patients at ICU, which is similar to the previous report from Iran from 2010 to 2012 in the western regions of Iran [21]. The mortality rates for infections caused by CRAB have been reported as 46% by Sheng et al. [22]. Patients hospitalized at ICUs in Asian and European countries more likely to get infection with resistant *A. baumannii* isolates than patients in the United States [23]. In this study, the respiratory tract of patients was the main organ that CRAB caused infection. This is similar to results reported by Sohail et al. [16] in Pakistan in 2016. Lee et al. [24] expressed that prolonged use of mechanical ventilators in ICU probably causes the stability of CRAB, leading the CRAB isolates as predominant bug in the respiratory tract.

In the last years, carbapenems were the choice for treatment against MDR *A. baumannii*. However, the extreme use of these antibiotics has converted them as resistant organism to various antibiotics. Higgins et al. [25] in 2009 showed that among the 515 *A. baumannii*, 471 isolates were resistant to imipenem, 21 were intermediate, and 23 were susceptible.

This study demonstrated that the annual incidence of CRAB has increased during 2014–2016. In Hong Kong, the rate of CRAB increased from 2.6% in 1997 to 29.4% in 2008 [26]. The rate of imipenem-resistant *Acinetobacter* in a Spanish hospital changed from 0 in 1991 to 50% in 2001 [27]. The global increase rate for imipenem-resistant *Acinetobacter* during 2000–2004 is 40% [16]. In this study, the MIC_{50} and MIC_{90} values for imipenem were 1,280. The MIC_{50} and MIC_{90} for meropenem were 80 and 320, respectively.

Based on the results of this study, the CRAB isolates were resistant to the majority of tested antibiotics. A significant increase in antimicrobial resistance has occurred worldwide from 2004 to 2009 [23]. In this study, 100% of CRAB was MDR and XDR. Therefore, the resistance rates of *A. baumannii* in this study were 99% against piperacillin/tazobactam, gentamicin, amikacin, ciprofloxacin and 98% to ampicillin/sulbactam and 97% against ceftazidime and trimethoprim/sulfamethoxazole, and 92% to tobramycin (85%). In a study from Spain conducted in 2014, all CRAB isolates were found resistant to piperacillin/tazobactam, gentamicin, imipenem, meropenem, doripenem, and ciprofloxacin, and majority of them were not susceptible to trimethoprim/sulfamethoxazole (95%), tobramycin (85%), amikacin (80%), and ampicillin/sulbactam (70%). In the same study, 50% and 20% were not susceptible to minocycline and tigecycline, respectively [13].

Colistin, polymyxin B, and tigecycline are the only available antimicrobial agents against the CRAB [29]. This study showed polymyxin B and tigecycline are effective against CRAB. In Turkey, tigecycline has a potent effect on MDR *A. baumannii* [29]. Colistin has more effect on the CRAB, but two isolates of CRAB were resistance to colistin in this study. This means that CRAB can acquire resistance to colistin. Maraki et al. [2] showed an increase of 7.9% resistance to

colistin during 2010–2014. Previous exposure of CRAB to colistin is important factor for acquiring resistance to this drug [13].

Combination therapy against infection with the CRAB is probably a good choice, for example, the bactericidal effect of polymyxin combined with tigecycline, rifampicin, or meropenem to XDR *A. baumannii* was promising [30]. Colistin combination with other agents, such as tigecycline, ampicillin/sulbactam, rifampin, and carbapenems, is a good combination for CRAB treatment. García-Quintanilla et al. [13] in 2014 observed lower mortality among patients who received colistin and carbapenem and ampicillin/sulbactam together compared with other regimens.

In this study, the $bla_{\text{OXA-23 like}}$ and $bla_{\text{OXA-24 like}}$ genes were abundant among the isolates (81% and 22%, respectively). Although we have previously found $bla_{\text{OXA-58 like}}$ gene in another study [20]; however, none of the isolates in this study contained this gene. In China, the MDR *A. baumannii* contained the $bla_{\text{OXA-23 like}}$ and $bla_{\text{OXA-58 like}}$ genes with the frequencies of 73% and 2%, respectively [26]. In India, Vijayakumar et al. [31] in 2016 demonstrated the $bla_{\text{OXA-51 like}}$ and $bla_{\text{OXA-23 like}}$ among all the 103 studied CRAB isolates. There is a relationship between the existence of $bla_{\text{OXA-23 like}}$ and resistance of *A. baumannii* to carbapenems [16]. In Brazil, CRAB is generally associated with carbapenemase production of $bla_{\text{OXA-23}}$ that is also reported in other countries [28]. In accordance with results of this study, the stewardship programs including molecular typing of bacteria should be performed to strengthen infection control to prevent further dissemination of CRAB.

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Conflict of Interest

None.

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