Antibiotic Resistance Patterns and Distribution of Ampc β-Lactamases Genes Among *Acinetobacter Baumannii* Clinical Isolates from Hospitals of Tehran, Iran

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Abstract

Background

Acinetobacter is a Gram-negative coccobacilli bacterium that can produce severe and different infections. Among the species of these bacteria, *Acinetobacter baumannii* is the most common cause of nosocomial infections. Due to the high prevalence of antibiotic resistance in this bacterium and the significant increase in antibiotic resistance, this study was conducted to investigate the antibiotic susceptibility of *A. baumannii* isolates from Iran. A total of 60 *A. baumannii* bacteria were isolated from the different clinical samples in hospitals of Tehran, Iran. The isolates susceptibility to 13 commonly used antibiotics was examined according to the Clinical & Laboratory Standards Institute (CLSI) guidelines. Using PCR, three important AmpC β-lactamases relayed genes (DHA, CIT and MOX) were detected.

Results

The highest and lowest resistance rate was related to ampicillin (98.3%) colistin (35%), respectively. Of 60 isolates, 59 isolates (98.34%) were resistant to more than 8 antibiotics. The frequencies of DHA, CIT and MOX genes were 1 (2%), 7 (12%), 27 (46%), respectively. Based on definition, 59 (88.33%), 44 (73.33%) and 13 (21.66%) isolates were MDR, XDR and PDR, respectively. Twenty-four isolates (40%) were negative for all three genes. There was a significant relationship between the presence of MOX gene and antibiotic resistance.

Conclusions

The high resistance rates of the *A. baumannii* isolates reported in the present study is alarming and need the management of treatment such as performing of antibiogram test before antibiotic therapy for select the appropriate antibiotic and also completes the course of treatment period.

Background

Acinetobacter is a non-motile, ubiquitous Gram-negative bacterium with the ability to grow on different laboratory culture media [1]. This opportunistic pathogen can colonize on the skin and mucosal membranes of the respiratory system of infected individuals [2]. Different species of this genus can cause various nosocomial infections, commonly among patients in intensive care units (ICUs) and high-dependency units (HDUs) [3]. *Acinetobacter baumannii* is more prevalent than other strains of genus Acinetobacter, and it is the most common cause of bacterium-induced infections [4]. Ventilator-associated pneumonia (VAP), septicemia, secondary meningitis, endocarditis, infections of the skin, soft tissues, urinary tract, and wound infection can be caused by bacteria *A. baumannii* bacteria [5].

To treatment of *A. baumannii* infections, various antibiotics, including β-lactams, fluoroquinolones, and aminoglycosides, are used. Today, many studies have shown that antibiotic resistance in the *A. baumannii* isolates is extremely increasing [3, 4, 6, 7]. Inappropriate and indiscriminate use of available antibiotics, high ability of natural genetic transformation in *A. baumannii* bacteria and its potential for widespread dissemination are most important factors contributed to the resistance of this pathogen to the various antimicrobial agents [8, 9].

Currently, β-lactam antibiotics play critical roles in the treatment of *A. baumannii* infections. But from 1991 until now there are many studies that reported the resistance of *A. baumannii* to this class of antibiotics [5]. Molecularly, resistance to β-lactams in *A. baumannii* may be due to the activity efflux pumps, changes in penicillin-binding proteins (PBPs), and the production of antibiotic-degrading enzymes such as production of β-lactamases [10].

Members of Gram-negative bacteria can hydrolyze many β-lactam antibiotics through the production of one or both of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases [11]. AmpC β-lactamases are clinically significant because they may confer resistance to a wide variety of β-lactam antibiotics, including α-methoxy-β-lactams, such as cefoxitin, narrow-, expanded-, and broad-spectrum cephalosporins, β-lactam-β-lactamase-inhibitor combinations, and aztreonam. AmpC β-lactamases are poorly inhibited by clavulanic acid; however, they are inhibited by cloxacillin [12]. AmpC β-lactamases is encoded by chromosomal or plasmid genes. Plasmid-mediated include MOX-, CIT-, DHA-, ACC-, FOX-, and EBC-type enzymes, and CMY-2 of CIT-type enzymes has shown the broadest geographic spread and is one of the main causes of β-lactam resistance at present [13].

According to these facts the present study was aimed to evaluate the phenotypic and genotypic antibiotic resistance pattern of *A. baumannii* clinical isolates and comparison of results to the previous described findings. In addition, we investigated the presence of AmpC β-lactamases enzyme production and also AmpC β-lactamases related genes.

Results

Demographic data

A total of 60 *A. baumannii* isolates were isolated from 240 clinical isolates were obtained based on applied differential and biochemical tests. These isolates were obtained from 27 (45%) women and 33 (55%) men. Among them, 6 (10%) cases hospitalized due to abdominal surgery, 7 (11.66%) cases due to heart surgery, 2 (3.33) cases due to brain surgery and 11(18.33%) cases due to respiratory problems. These data are presented in detail in Table 1.

Table 1
Clinical characteristics of patients infected with *A. baumannii* isolates

Cause of hospitalization	Women	Men	Total
abdominal surgery	2 (3.33%)	4 (6.66%)	6 (10%)
heart surgery	3 (5%)	4 (6.66%)	7 (11.66%)
brain surgery	1 (1.66%)	1(1.66%)	2 (3.33%)
respiratory problems	4 (6.66%)	7 (11.66%)	11 (18.33%)
heart problems	3 (5%)	6 (10%)	9 (15%)
cancer	2 (3.33%)	6 (10%)	8 (13.33%)
premature infant	1 (1.66%)	1 (1.66%)	2 (3.33%)
stroke	0	2 (3.33%)	2 (3.33%)
diabetes	1 (1.66%)	1 (1.66%)	2 (3.33%)
pelvic surgery	1 (1.66%)	1 (1.66%)	2 (3.33%)
brain problems	3 (5%)	0	3 (5%)
plastic surgery and liposuction	4 (6.66%)	0	4 (6.66%)
aortic valve surgery	1 (1.66%)	0	1 (1.66%)
parturition	1 (1.66%)	0	1 (1.66%)
Total	27 (45%)	33 (55%)	60 (100%)

Antibiotic resistance patterns

Our results revealed that out of 15 studied antibiotics, the resistance rate above 90% was reported in 12 cases. Based on our results the tested isolates are highly resistant to the ampicillin (99%), cefotaxime (98%), chloramphenicol (98%), ceftriaxone (98%), ceftazidime (98%), meropenem (98%) and ticarcillin (98%). On the other hand, tobramycin and colistin with 78.3% and 35% resistance rates were the most effective antibiotics, respectively. The results of the present study showed high resistance rate of clinical isolates to tested antibiotics. The total resistance pattern was presented in Table 2. In this study, out of 60 isolates, 59 isolates (98.34%) were resistant to more than 8 antibiotics and only 1 sample (1.66%) which isolated from urine culture was sensitivity to all studied antibiotics.

Table 2
Antibiotic resistance pattern of *A. baumannii* isolates

Antimicrobial category	Antimicrobial agent	Number (Frequency)		
		R	I	S
Aminoglycosides	gentamicin	57 (95)	0	3 (0.5)
	amikacin	47 (78.3)	0	13 (21.7)
	tobramycin	47 (78.3)	0	13 (21.7)
Cephalosporins	cefotaxime	58 (96.7)	0	2 (3.3)
	ceftriaxone	58 (96.7)	0	2 (3.3)
	ceftazidime	58 (96.7)	1 (1.7)	1 (1.7)
	cefepime	55 (90)	1 (1.7)	4 (6.7)
Carbapenems	meropenem	58 (96.7)	0	2 (3.3)
Penicillins	ampicillin	59 (98.3)	0	1 (1.7)
	piperacillin	55 (90)	1 (1.7)	4 (6.7)
	ticarcillin	58 (96.7)	0	2 (3.3)
Fluoroquinolones	ciprofloxacin	56 (93.3)	0	4 (6.7)
Sulfonamide	Trimethoprim/sulfamethoxazole	56 (93.3)	0	4 (6.7)
Chloramphenicol	chloramphenicol	58 (96.7)	0	2 (3.3)
Polymyxins	colistin	21 (35)	2 (3.3)	

MDR, XDR and PDR isolates

Based on definition of tested isolates, 59 isolates (88.33%) were in the MDR group, where 44 isolates (73.33%) were XDR. Our results showed that 13 isolates (21.66%) were PDR.

Distribution of Amp β-lactamases genes

The prevalence of DHI, MOX and CIT were 1 (1.66%), 27 (45%) and 7 (11.66%), respectively. Twenty-four isolates (40%) were negative for all three genes. There was a significant relationship between the presence of MOX gene and antibiotic resistance ($p < 0.05$). In other word, MOX-containing isolates were resistance to more tested antibiotics.

Discussion

In the past 2 decades, *A. baumannii* has become an important pathogen related to nosocomial infections and has been shown to increase morbidity and mortality. Control of infections caused by this bacteria is always difficult, due to its acquisition of multidrug-resistant phenotypes, such as resistance to fluoroquinolones, aminoglycosides, cephalosporins, and carbapenems [1].

In the present study, the antibiotic resistance profile as well as the phenotypic and genotypic AmpC β-lactamases resistance was investigated. Our results revealed that out of 15 studied antibiotics, 12 antibiotics had a resistance above 90%. The most resistance rate was related to ampicillin (99%), cefotaxime (98%), chloramphenicol (98%), ceftriaxone (98%), ceftazidime (98%), meropenem (98%) and ticarcillin (98%). These results can be more important in related to the antibiotics which are used to treatment of *A. baumannii* infections; such as third- and fourth-generation cephalosporins and carbapenems [2].

The results of this study are in line with the findings of previous studies which reported that most strains of *A. baumannii* isolated in Iran were resistant to first-line drugs including aminoglycosides, fluoroquinolones and carbapenems [14–16]. In a study conducted by Karimi et al (2020) on 60 *A. baumannii* isolates obtained from Hazrat-e-Rasoul Hospital in Tehran, the highest resistance of isolates was related to ceftazidime (93.3%) and amikacin (91.6%), respectively. In their study, colistin with 3.3% resistance was introduced as the most effective antibiotic [17]. In terms of antibiotic resistance profile, the results of this study were in line with our present study.

Due to rising antimicrobial resistance, carbapenems are the cornerstone of therapy for the treatment of *A. baumannii* serious infections [18]. However, the results of the present study showed a high prevalence of meropenem resistance (96.7%), which has increased significantly compared to previous studies in different cities of Iran and also other countries [19, 20]. In similar studies in Tehran, 50.9%, 52.5%, 62% and 67.5% of the isolates were resistant to imipenem and 51.8%, 52.5%, 62% and 84.5% to meropenem in 2008, 2009, 2011 and 2013, respectively [16, 21–23].

The analysis of these data and comparing them with the results of the present study results reveals that antibiotic resistance of *A. baumannii* isolates to carbapenem antibiotics were increasing in Iran over the time.

In our study low susceptibility rates to most of available antimicrobial agents for the treatment of *A. baumannii* isolates was seen, except for colistin. Although several number of previous studies conducted in Iran have shown that all of the *A. baumannii* isolates were sensitive to colistin [15, 21, 24], but in the present study, 35% of the isolates were resistant to colistin. Given that drug resistance rate of *A. baumannii* to colistin was not high, this antibiotic can be administered as appropriate therapeutic drug against *A. baumannii* infections.

Based on our results, 59 (88.33%), 44 (73.33%) and 13 (21.66%) isolates were MDR, XDR and PDR, respectively. Based on studies, emergence of MDR, XDR and PDR of *A. baumannii* strain are currently reported [25].

In contrast to the present study which found PDR and XDR as well as MDR *A. baumannii* isolates, Hojabri (2014) only found MDR strains among the isolates [26]. In Bahador et al (2015) study, the frequency of MDR, XDR and PDR isolates were 69, 24, and 0%, respectively[27]. In similar study performed by Sobouti et al (2020) in Iran, from 62 *A. baumannii*, 36 (58%) strains were categorized as MDR, 17 (27.5%) as XDR, and 9 (14.5%) as PDR [25]. Compared to the results of this study, the percentage of resistant strains in the present study was higher. Comparison of these results and also similar studies shows that the emergence of resistant strains in *A. baumannii* isolates is increasing in Iran and the strict control measures should be considered in this subject.

In next part of study the prevalence of some AmpC β-lactamases related genes (FOX, DHA and MOX) in *A. baumannii* isolates were detected. Based on these results, it was found that out of 60 isolates, 1 (1.66%), 27 (45%) and 7 (11.66%) isolates were positive for DHI, MOX and CIT, respectively. Consistent with the present study, in Fekri et al (2017) study AmpC β-lactamases related genes detected in 60 strains *A. baumannii* isolates using Multiplex PCR, of which 39 (65%) had CIT, 36 (60%) had DHA and 12 (20%) had MOX gene [28]. The products of these genes were consistently associated with resistance or at least reduced susceptibility to a wide variety of β-lactam antibiotics. For this reason the presence of these genes in the individual isolate is very important [29]. Our results also shown that, there is some cephalosporins-resistant isolates which are negative for the some investigated genes. Resistance to these antibiotics in those isolates may be due to other mechanisms including decreased permeability, alteration of PBPs, presence of other AmpC related genes and overexpression of efflux pumps [30].

Conclusion

This study has shown that resistance to the majority of antibiotics in the *A. baumannii* strains is high with 59 (88.33%), 44 (73.33%) and 13 (21.66%) isolates were MDR, XDR and PDR, respectively. High rate of resistance to cephalosporins has been seen among our isolates and it seems that, colistin can be effective drug for treatment of *A. baumannii* infections. Progressive increase in resistance to the majority of antibiotics and multiple resistances in the present study may be related to increased usage of these antibiotics for treatment of *A. baumannii* infections. Management of treatment, such as performing of antibiogram test before antibiotic therapy for select the appropriate antibiotic and also completes the course of treatment period is necessary to prevent the further spread of resistant isolates.

Methods

Bacterial isolation

A total of 240 clinical samples (including blood, urine, sputum, respiratory secretions, urine, wounds and skin) were collected from patients admitted to different wards of hospitals in Tehran between 2018 to 2020. Samples were collected from patients who had been hospitalized for at least three days and who had received the infection from the hospitals. *A. baumannii* were identified using Gram staining, culture characteristics on the differential culture media (blood agar, MacConkey agar, Triple sugar iron agar (TSI)

and oxidative-fermentative (OF) agar and biochemical tests (catalase and oxidase) [31]. The isolated bacteria were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 30% glycerol at -70°C until further analysis.

Antibiotic Susceptibility Testing

The antibiotic susceptibilities testing for *A. baumannii* isolates were done by Kirby Bauer's disk diffusion method on Muller-Hinton agar (Merk, Germany) according to the CLSI [32]. The applied antibiotic disks (MAST, UK) were ampicillin (25 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), meropenem (10 µg), ticarcillin (75 µg), gentamicin (30 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (75 µg), cefepime (30 µg), piperacillin (100 µg), amikacin (30 µg), tobramycin (30 µg), colistin (25 µg).

Determination of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) isolates

According to the CLSI, MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories for example bacterial isolates remain susceptible to only one or two categories and PDR was defined as non-susceptibility to all agents in all antimicrobial [33].

Dna Extraction And Multiplex Pcr

DNA extraction was carried out from 10 ml overnight cultures of each isolate in tryptic soy broth (TSB) by Cinnagen extraction kit (Cinnagen, Iran) according to the manufactory instructions. Extracted DNA samples from all of the isolates were examined by the PCR assay for three target genes, *MOX*, *CIT* and *DHA*.

The oligonucleotide primers were previously described by Hanson et al [34] and their sequences are given in Table 3. For each gene, the final volume of the reaction mixture was 25 µl contained 3 µl of template DNA, 2.5 µl of ×10 PCR buffer, 0.5 µl of 10 mM dNTPs, 0.75 µl of 50 mM MgCl2, 0.25 µl of 5 U/µl of Taq DNA polymerase, and 25 pmol of each used primer.

The PCR amplification was performed under the following thermal conditions: initial denaturation at 94°C for 1 min followed by denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1.5 min (30 cycles), and a final extension at 72°C for 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel containing 0.5 µg/ml ethidium bromide.

Table 3
Sequence of primers used to screen studied genes

Target	Primer	Sequence (5' to 3')	Size (bp)	Reference
MOX	MOX -F	GCTGCTCAAGGAGCACAGGAT	520	[34]
	MOX -R	CACATTGACATAGGTGTGGTGC		
CIT	CIT-F	TGGCCAGAACTGACAGGCAAA	462	
	CIT-R	TTTCTCCTGAACGTGGCTGGC		
DHA	DHA-F	AACTTTCACAGGTGTGCTGGGT	246	
	DHA-R	CCGTACGCATACTGGCTTTGC		

Abbreviations

PCR: Polymerase chain reaction; *A. baumannii*: *Acinetobacter baumannii*; CLSI: Clinical & Laboratory Standards Institute; MDR: Multidrug-resistant; XDR: Extensively drug-resistant PDR: Pandrug-resistant; ICU: Intensive care units; HDU: High-dependency units; VAP: Ventilator-associated pneumonia; PBPs: Penicillin-binding proteins; ESBLs: extended-spectrum β-lactamases; TSI: Triple sugar iron agar; OF: Oxidative-fermentative; TSB: Tryptic soy broth.

Declarations

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Authors' contributions

MB designed the study wrote the manuscript. SA does laboratory tests. NB analysis the results. All authors read and approved the manuscript.

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Availability of data and materials

All data generated during this study are included in this published article.

Ethics approval and consent to participate
Ethics statement

This research was approved by the Ethical Committee of Islamic Azad University, Kazerun Branch, Kazerun, Iran (Code No: IR.IAU.KAU.REC.1399.104). In addition, informed consent form was obtained from all of the patients.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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