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Antibiotic resistance patterns among *Acinetobacter baumannii* strains isolated from burned patients

Maryam Pourhajibagher[1], Masoumeh Mokhtaran[1], Davood Esmaeili[2] and Abbas Bahador[3,4,1]*

[1]*Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Applied Microbiology Research center, and Microbiology Department, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[3]*Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Laser Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran*

ABSTRACT

Emergence and spread of Acinetobacter baumannii infections and resistance to most of the antibiotics are a global concern. Recently, we are facing with the development of multi drug resistance (MDR) A. baumannii. Since the organism causes outbreaks of infection and health care associated, the appropriate antibiotic choice for the treatment is a priority. This study was performance in order to elucidate the antibiotic resistance trends among A. baumannii strains. A total of 120 non-duplicate isolates recovered from patients with burn wounds were subjected to conventional cultural and biochemical tests. For those isolates that were preliminary identified as A. baumannii, multiplex PCR was performed. Antimicrobial susceptibility testing was done by disk diffusion agar and broth microdilution methods. In total, 100 isolates (88.3%) were identified as A. baumannii using conventional phenotypic methods with subsequent confirmation by multiplex PCR. The majority of the rates of antibiotic susceptibility in A. baumannii were belonged to colistin, tigecycline, tetracycline, and ampicillin/sulbactam with 99%, 81%, 71%, and 56%, respectively. High levels of resistance to beta-lactam antibiotics and cephalosporinswere found in our isolates. Among other isolates, MDR A. baumanniistrains showed the most susceptibility to colistin, tigecycline, ampicillin/sulbactam, tetracycline, and imipenem. Combinations antimicrobial agents and prevention of infections transmission are essential in controlling MDR A. baumannii outbreaks, especially in developing countries such as Iran.

Key words: *Acinetobacter baumannii*, multi drug resistance, antibiotic, Iran

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INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen with increasing relevance in community-acquired and nosocomial infections [1]. *A.baumannii* has been implicated in diverse infections, including endocarditis, secondary meningitis, ventilator-associated pneumonia (VAP), septicemia, infections of the skin, soft tissues, and urinary tract, abdominal abscesses, and surgical wound infections [2-5].

Prolonged length of hospital stay, presence of susceptible patients, exposure to an intensive care unit (ICU), colonization pressure, exposure to antimicrobial agents and antibiotics, and incomplete compliance with infection control procedures are some of the reasons for the emergence of antibiotic resistance against *A. baumannii* [4, 6, 7]. *A. baumannii* infections were impressively treated with traditional antibiotics in about three decades ago [8]. By contrast, nowdays it displays resistance to approximately all main classes of antibiotics, including broad-spectrum penicillins, chloramphenicol, fluoroquinolones, cephalosporins, carbapenems, aminoglycosides, and tetracyclinees [4, 8].

Rapid emergence of resistance to several antibiotics, increased incidence, and the universal spread of multi drug resistance (MDR) isolates are the troubling evolution [9].

Widespread outbreaks of MDR (the isolate that is resistant to at least one agent in three classes of antimicrobial groups), extensive drug resistant (XDR; the isolate that is resistant to at least one agent in all but two or fewer antimicrobial categories), and pandrug resistant (PDR; XDR isolate that is resistant to polymyxins and tigecycline) *A. baumannii* infections have further limited effective choices for the treatment of *A. baumannii* infections [4].

In these circumstances, find the best antibiotic treatment is important. Combination antibiotic therapy is a strategy often employed in the treatment of MDR *A. baumannii* infections [10].The current study was performed to elucidate the trends of antibiotic resistance of *A. baumannii* isolates to several classes of antibiotics.

MATERIALS AND METHODS

Bacterial isolates

A total of 120 nonduplicate isolates from patients with burn wounds were collected from Motahari hospitals in Tehran, Iran, from Oct 2012 toJun 2013.

Species identification

The isolates were identified as Acinetobacter spp. based on the preliminary results of conventional biochemical tests which determine the phenotypic characteristics including growth on MacConkey agar, catalase and oxidase tests, sugar fermentation, motility, and other standard recommended tests [11, 12]. In the following, molecular methods were used for definitive identification of these isolates.

Molecular methods

A. baumannii genomic DNA was prepared from fresh overnight cultures grown in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) at 37°C as described previously [13]. Extracted DNA was resuspended in 100 µl of TE buffer (10 mMTris, 1 mM EDTA [pH 8.0]) and boiled 15 min. Purified DNA was aliquoted and stored at −20°C. *A. baumannii* strains were identified using species-specific *gyrB* gene-based multiplex PCR as described previously [14]. Primer sequences are shown in Table 1. The PCR amplicons obtained were submitted to electrophoresis in 1% agarose gel then were stained with ethidium bromide (0.5 µg/ml) for UV light analysis and digitized (UVIDOC-CF08.XD).

Table 1: Multiplex PCR primers for detection of *A. baumannii*

Primers	Sequence (5′ to 3′)	Reference
gyrB-2	CTTCCGACGCGTCATTTCAC	14
D14	GACAACAGTTATAAGGTTTCAGGTG	
D19	CCGCTATCTGTATCCGCAGTA	
D16	GATAACAGCTATAAAGTTTCAGGTGGT	
D8	CAAAAACGTACAGTTGTACCACTGC	
Sp2F	GTTCCTGATCCGAAATTCTCG	
Sp4F	CACGCCGTAAGAGTGCATTA	
Sp4R	AACGGAGCTTGTCAGGGTTA	

Antimicrobial susceptibility testing by disk diffusion method

The antibiotic susceptibilities of clinical isolates were determined by Kirby Bauer's disk diffusion method on Muller-Hinton agar (Merk, Germany) according to the Clinical and Laboratory Standards Institute (CLSI) criteria [15]. The antibiotic disks (MAST, UK) applied were cefepime (CPT; 30 µg), ceftriaxone (CRO; 30µg), cefotaxime

(CTX; 30 µg), piperacillin (PIP; 30 µg), piperacillin/tazobactam (PTZ; 100 + 10 µg), ceftazidime (CAZ; 30 µg), ticarcillin (TIC; 75 µg), meropenem (MEM;10µg), gentamycin (GM; 10 µg),ciprofloxacin (CIP; 5 µg),amikacin (AMK; 30 µg), tobramycin (TOB;10µg),imipenem (IPM; 10 µg), ampicillin/sulbactam (SAM; 10 + 10 µg), tetracycline (TET; 30 µg), and tigecycline (TGC; 15 µg).*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. They were incubated at 37°C for 18- 24 hours. The diameter of the zone of inhibition was measured and compared to that of standard strain.

Determination of minimum inhibitory concentration (MIC) for colistin

MIC of colistin (Sigma, Germany) against *A. baumannii* was determined by broth microdilution method as recommended by CLSI. 50 µl from final concentrations of colistin (512 µg/ml) was prepared and added to each wells of single 96-well round-bottomed sterile polystyrene microplate (TPP; Trasadingen, Switzerland) containing 50µl Mueller Hinton broth (CAMHB; Himedia, India). Colistin concentration will be diluted this way 1:2 in range from 512 µg/ml to 1 µg/ml. Using the multi-channel pipet, 50 µl/well of fresh CAMHB bacterial cultures adjusted to a concentration of 1.0×10^6 CFU/ml were then added. The final bacterial concentration in the wells was 1.0×10^5 CFU/ml. Colistin-free medium was used in control well. Then microplate was incubated at 37°C. After 24 h of incubation, MIC was determined as the lowest concentration of colistin at which visible bacterial growth was significantly inhibited.

Statistical analysis

Data from the experiments was evaluated using SPSS ver. 22.0 (SPSS Inc., Chicago, IL, USA) and $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

During the study, 120 clinical isolates suspected to *A. baumannii* were collected that 100 isolates of them identified as *A. baumannii*by conventional biochemical and molecular assessments, which represented 83.3% of all the isolated strains.Table 2 summarized the results of theantibiotics susceptibility tests of *A. baumannii* strains.

All of 100 isolates of *A. baumannii* were resistant to 17 different antibiotics, belonging to eight different classes of antibiotic. According to CLSI antimicrobial susceptibility testing standards, the majority of the rates of antibiotic susceptibility in *A. baumannii* were belonged to colistin (99%), tigecycline (81%), tetracycline (71%) and ampicillin/sulbactam (56%). These isolates had resistance between 59- 98% to other antibiotics.

As can be seen in Table 2, the high levels of resistance (> 90%) were found in the group of beta- lactam antibiotics (such as penicillin and cephalosporins).

Table 2: Resistance rates of *A. baumannii* isolates to antimicrobial agents

Samples (100)	FEP	CRO	CTX	PIP	PTZ	TIC	CAZ	MEM	GEN	CIP	AMK	TOB	IPM	SAM	TET	TGC	CL
	Number (%)																
Frequency (%)	99	98	98	95	93	93	94	91	90	89	78	63	60	44	29	19	1
None	3 (75)	2 (50)	1 (25)	0	0	0	1 (25)	4 (100)	0	0	0	0	4 (100)	0	0	0	0
MDR	33 (100)	33 (100)	33 (100)	33 (100)	3 (94)	31 (93)	30 (91)	28 (85)	30 (91)	27 (82)	(63)	2 (69)	4 (12)	10 (30)	30 (91)	2 (6)	0
XDR	62 (100)	62 (100)	62 (100)	61 (98)	61 (98)	61 (98)	62 (100)	62 (100)	59 (95)	61 (98)	(90)	39 (62)	55 (89)	34 (55)	59 (95)	17 (27)	0
PDR	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	0	1 (100)

CPT (Cefepime), Ceftriaxone (CRO), Cefotaxime (CTX), Piperacillin (PIP), Piperacillin/Tazobactam (PTZ),Ceftazidime (CAZ), Meropenem (MEM), Gentamycin (GM), Ciprofloxacin (CIP), Amikacin (AMK), Tobramycin (TOB), Imipenem (IPM), Ampicillin/Sulbactam (SAM), Tetracycline (TET), Tigecycline (TGC), Colistin (COL). MDR: Multi Drug Resistant, XDR: Extremely Drug Resistant, PDR: Pan Drug Resistant.

In this study, 62%, 33%, 4% and 1% of the 100 isolates had XDR, MDR, Non MDR- XDR, and PDR phenotypes. MDR*A. baumannii* strains showed the most susceptibility to colistin, tigecycline, ampicillin/sulbactam, tetracycline, and imipenem and PDR strain was resistant to colistin(MIC≥ 32µg/ml).

DISCUSSION

A. baumannii infection has become a serious challenge to global health care systems and management of it is a great perturbation and common problem for physicians and clinical microbiologists [1, 2]. During the past decade, antimicrobial resistance among *A. baumannii* has increased [4].

Multiple mechanisms have been found to be responsible for the resistance to antibiotics in *A. baumannii* that generally falls into three broad groups: (1) antimicrobial inactivating enzymes, (2) decrease access to microbial targets, and (3) mutations [16]. *A. baumannii* has a broad array of beta lactamases enzymes that can hydrolyze the beta lactam antibiotics and resistance to cephalosporins, carbapenems, and penicillins when expressed [17]. On the other, the loss or decreased expression of porin channels, alterations in the structure and number of porin proteins, and mltidrug efflux pumps that are capable of actively removing a wide range of antimicrobial agents from the bacterial cell which could potentially disrupt the cytoplasmic membrane, lead to reduced outer membrane permeability that cause the resistance to antibiotics such as carbapenem [18]. Also, change of targets or upregulating cellular functions (alterations in penicillin binding proteins) due to mutations such as point mutations is another mechanism of resistance [19].

The appropriate antibiotic choice is essential for treatment of *A. baumannii* infections and is guided foremost by in vitro antimicrobial susceptibility tests [20]. Among these, the determination of MICs by broth microdilution has been considered the “gold standard” [21]. On the other hand, the reliability and comparability of susceptibility testing such as disk diffusion agar or the Etest have been also reconciled for *A. baumannii* [20].

As mentioned, our data show that colistin, tigecycline, and tetracycline had the less rate of resistance against *A. baumannii* , respectively. On contrary, cephalosporins (including cefepime, ceftriaxone, cefotaxime, ceftazidime), piperacillin/tazobactam, and ticarcilin showed the most rates of resistance against *A. baumannii* isolates, respectively.

Often colistin or tigecycline are the only available treatments for MDR *A. baumannii* infections [22]. Monotherapy is not recommended for severe *A. baumannii* infection. Formerly, treatment of *A. baumannii* infection included a beta-lactamase-stable beta-lactam such as piperacillin or imipenem, in combination with aminoglycosides, such as amikacin. Montero et al. [23] found that the combinations of rifampin with imipenem, tobramycin, or colistin were the most effective regimens against MDR *A. baumannii*. Pourhajibagher et al. [4] stated that the combinations of imipenem with rifampicin, tigecycline and colistin are recommended as the best therapeutic approach for treatment of nosocomial infections of *A. baumannii*due to their effectiveness and low toxicity. Owen et al. [24] also found that combination therapy may be advisable to prevent the emergence of colistin resistance during monotherapy.

In addition to an increase in antibiotic resistant *A. baumannii* strains from 2001 to 2013 in Iran, the prevalence of MDR strains also increased (from 50% in 2001-2007 to 74% in 2010-2011), with a mean prevalence of 71.2% [25]. Pourhajibagher et al. [4] reported that 55% of *A. baumannii*were resistant to imipenem and 74% were MDR.

Treatment of MDR strains is usually difficult. Several studies revealed that colistin can cure or improve the 57%-77% of patients with MDR *A. baumannii* infections [26-29]. Other studies have reported more favorable clinical response rates (56%-61%) for parenteral colistin treatment of MDR Acinetobacter VAP [30-33]. In our study, colistin and tigecycline showed the less rates of MDR and XDR phenotype compared with other antibiotics.

CONCLUSION

Notwithstanding a background for relatively low virulence, MDR *A. baumannii* infection poses a terrible threat to patients. The significant health challenges for treatment of *A. baumannii* and selection of the best antibiotics are exacerbated by prolonging hospitalization, treatment failures, and increased mortality. To the best of the authors’ knowledge, no controlled trials to guide therapeutic choices.

Antimicrobial susceptibility test is important in providing useful information for effective treatment, and occasionally more than one antibiotic is required to cure and improve *A. baumannii* infections. However, antibiotic treatments are not always the same for the difference of medical cognition in different regions.

However, based on the results of this study, colistin in combination with tigecycline are useful antibiotic compounds for *A. baumannii* strains isolated from patients with burn wounds. Nevertheless, the gaps in the current knowledge of the response and bacterial mechanisms of antimicrobials resistance exist and the critical need for a comprehensive monitoring and infection control policy MDR *A. baumannii* isolates from various parts of Iran is noteworthy.

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