



## Antimicrobial resistance and molecular typing of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from a burn hospital in Tehran, Iran

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### Abstract

**Background and Objective:** *Acinetobacter baumannii* and *pseudomonas aeruginosa* are common opportunistic gram-negative bacteria related to hospital-acquired infections. Multidrug resistant microorganisms have emerged as the causes of nosocomial infections worldwide. In this study, we evaluated the existence of *blaTEM*, *blaSHV* and *blaCTX-M* genes among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from hospitalized patients in one burn hospital in Tehran, Iran.

**Materials and Methods:** From June 2015 to May 2016, 82 isolates were collected from burn patients hospitalized in one burn hospital in Tehran, Iran. A total of 82 isolates of gram-negative, non-fermentative bacilli including *pseudomonas aeruginosa* (58 isolates) and *Acinetobacter baumannii* (24 isolates) were tested for susceptibility to selective antibiotics by disk diffusion recommended in CLSI guidelines. All the resistant isolates were subjected to PCR assay for *blaTEM*, *blaSHV*, and *blaCTX-M* genes that encode ESBL.

**Results:** Resistance to gentamicin was 83%, but resistance to cephalosporins was higher than gentamicin. Out of the 47 *pseudomonas aeruginosa* resistance isolates, 24 and 9 isolates were CTX-M and TEM procedure, respectively. Among 8 *Acinetobacter baumannii* isolates that were resistant to all antibiotics, 7 and 1 isolates were CTX-M and TEM producer, respectively. In addition, *bla SHV* gene was not detected in any of the isolates.

**Conclusion:** High level of resistance to most antibiotics tested and high prevalence of *bla CTX-M* gene in this study, indicating the careful detection of antimicrobial resistant strains is needed in order to prevent further resistance to antimicrobial agents in Iran.

**Key words:** *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *bla CTXM*, *blaTEM*, *bla SHV*

### 1. Introduction

Many communities have been encountered with a growing epidemic of antibiotic resistant due to gram-negative bacteria (1). The burn wound has been considered one of the major problems due to bacterial infections. Burns provide a suitable place for bacterial infections. Prolonged period of staying of patients in burn wards and the considerable threat of the spread of multidrug-resistant bacterial pathogens, which cause nosocomial infections, are the main concerns of physicians. Despite recent improvement in burn patient care and

use of a wide variety of antimicrobial agents, multidrug-resistant microorganisms have increased (1,2).

*Acinetobacter baumannii* and *pseudomonas aeruginosa* are important opportunistic pathogens that can cause nosocomial infections in burn units and several resistance mechanisms have been identified in these two pathogens, including acquisition of resistance encoding genes through mobile genetic elements (3).

Beta-lactam antibiotics have been used in recent years for treating these infections and are powerful

agents for treating infections caused by these two pathogens. Recently, the susceptibility to these drugs has been mostly decreased (4). Resistance to beta-lactam antibiotics is more prevalent among opportunistic non-fermentative bacteria such as *A. baumannii* and *P. aeruginosa*. *Pseudomonas aeruginosa* develops antimicrobial resistance rapidly and is frequently isolated in burn patients (5). Although *pseudomonas aeruginosa* has wide distribution in nature, serious infections with *pseudomonas aeruginosa* are predominantly hospital-acquired (6). In recent years, *Acinetobacter baumannii* have acquired resistance to Beta-lactam antibiotics like secondary and third-generation cephalosporins because of their ability to produce Beta-lactamase enzymes which are inhibited by clavulanic acid (7). The significant problem associated with *Acinetobacter baumannii* is resistance to multiple antibiotics and its ability to rapidly acquire antibiotic resistance from other bacteria (8).

Extended spectrum beta lactamases (ESBLs) are one of the main leading causes of resistance to beta lactam antibiotics among Gram-negative bacteria. ESBLs are among the Ambler Class A beta lactamases, which can decompose monobactams and cephalosporins but not carbapenems or cephamycins. Also, there are several genotypes of ESBLs such as *bla*SHV, *bla*TEM, and *bla*CTX-M types (9). Therefore, the aim of the present study was to investigate screening of ESBL produced by *A. baumannii* and *P.aeruginosa* isolated from a burn hospital in Tehran, Iran.

## 2. Materials and Methods

### 2.1. Bacterial strains

From June 2015 to May 2016, 82 non-duplicate isolates of *A. baumannii* and *P.aeruginosa* were isolated from wound, blood and urine samples of patients from hospitalized cases in one burn hospital in Tehran, Iran.

Information about age, sex, and type of infection were obtained from medical records. Total of isolates were transferred to the Microbiology Department at the Medicine School of Shahed University for future studies.

The isolates were classified as *Pseudomonas aeruginosa* species based on: gram-negative bacilli, colonies with characteristic pigments (blue and green) and a special odor, positive catalase and oxidase tests, oxidation of glucose on OF medium, and growth in 42°C and isolates were classified as *Acinetobacter baumannii* species based on oxidative negative, non-fermenter and non-motile. *Acinetobacter baumannii* did not produce acid from mannitol, sucrose and H<sub>2</sub>S on TSI media and had no reaction on lysine decarboxylase medium. *A. baumannii* isolates were confirmed by *bla*OXA-51 gene PCR (10).

All clinical isolates of *pseudomonas aeruginosa* (58) and *Acinetobacter baumannii* (24) were identified by the standard microbiological methods. All identified isolates were stored in 15 percent glycerin trypticase soy broth and then frozen at 80°C prior to susceptibility testing.

### 2.2. Phenotypic determination of antibiotic resistance:

Disk diffusion tests for tetracycline (30 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), and ceftizoxime (30 µg) were performed in Mueller-Hinton agar (Merck, Darmstadt, Germany). For this purpose, Mueller-Hinton agar plates were inoculated with a bacterial suspension in saline of standardized density (optical density of 0.5 Mac Farland) that has been prepared from a 24 h culture on blood agar. Plates were incubated for 24 h at 37°C, after which inhibition zones were measured and the results were interpreted according to guidelines of the clinical and laboratory standards Institute CLSI (11), with *pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 as controls. Antibiotic disks were purchased from MAST Diagnostics (Merseyside, UK).

### 2.3. Genotypic detection of *bla* CTX, *bla* TEM, *bla* SHV by polymerase chain reaction (PCR)

Polymerase chain reaction was used to detect *bla* CTX-M, *bla* TEM and *bla* SHV genes among isolates which were resistant to selective antibiotics. In this procedure DNA was extracted by the boiling method (12). PCR was performed with primer specific for *bla* CTX, *bla* TEM, *bla* SHV genes (13-15). Primers were purchased from Bioneer (Korea) and their sequence, temperature cycle profiles and PCR fragment size are shown in Table 1. The total volume of PCR mix was 25 µl including sterile redistilled H<sub>2</sub>O (17.05 µl), 10X PCR buffer (2.5 µl), dNTP mix (10 mM) (0.5 µl), MgCl<sub>2</sub> (50 mM) (0.75 µl), forward primer (25 µM) (0.5 µl), reverse primer (25 µM) (0.5 µl), Taq DNA polymerase (5 U/µl) (0.2 µl), and template DNA (3 µl). Negative controls contained all components except template DNA. Reagents were prepared according to the manufacturer's recommendation.

In each PCR reaction, there was an initial denaturation step at 94°C for 2 minutes for *bla* CTX-M and 94°C for 12 minutes for *bla* TEM and *bla* SHV and a final extension step at 72°C for 3 minutes for *bla* CTX-M and at 72°C for 10 min for *bla* TEM and *bla* SHV genes. Then, 5 microliters of PCR product was used for agarose gel electrophoresis (1x Tris acetate, EDTA, 100 V (100 min)) and gel was stained with ethidium bromide. PCR segment was seen using a gel documentation system by comparing with a molecular size marker (100 bp ladder, Eurobio, UK).

**Table 1.** Primer features

Gene detected	Primers sequence	Amplicon size (bp)	References
<i>blaCTX-M</i>	5'-ATGTGCAGYACCAGTAARGT-3' 5'-TGGGTRAARTARGTSACCAGA -3'	544	(13)
<i>blaTEM</i>	5'-ATAAAATTCCTGAAGAC-3' 5'-TTACCAATGCTTAATCA-3'	1075	(14)
<i>blaSHV</i>	5'-AAGATCCACTATCGCCAGCAG-3' 5'-ATTCAGTCCGTTTCCCAGCGG-3'	230	(15)

#### 2.4. Statistical analysis

SPSS (v.11) for Windows was used to analyze the data and a Fisher exact test was used for the categorical data. P-value<0.05 was considered significant (two-tailed test).

### 3. Results

During the study period, 82 burn patients were admitted to the burn unit at Shahid Motahari hospital. A total of 82 isolates were obtained from wound (76%), blood (15%), and urine (9%) samples. Infections were observed more frequently in men (80%). Of 82 clinical specimens obtained, 58 were positive for *Pseudomonas aeruginosa* and 24 were positive for *Acinetobacter baumannii*. Resistant rate to various antibiotics are presented in Table 2.

Of the 58 *pseudomonas aeruginosa* isolates, 47 isolates were resistant to all antibiotics and of the 24 isolates that were *Acinetobacter baumannii*, 8 isolates were resistant to all antibiotics.

Out of the 47 *pseudomonas aeruginosa* resistant isolates, 24 and 9 isolates were CTX-M and TEM producers, respectively. While SHV gene was not found in none of the isolates.

Among 8 *Acinetobacter baumannii* isolates that were resistant to all antibiotics, 7 out of them were found to have CTX-M gene and only 1 out of them was found to have TEM gene while none of them have not SHV gene.

**Table 2.** Antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates

Antimicrobial agent	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>
	N - R	N - R
Tetracycline	57 - 98.2%	8 - 33%
Gentamicin	48 - 82.75%	20 - 83%
Amikacin	49 - 84.5%	23 - 96%
Ciprofloxacin	51 - 88%	23 - 96%
Ceftriaxone	52 - 90%	24 - 100%
Ceftizoxime	56 - 96.5%	23 - 96%

N= number of resistant isolates

R= Percentage of resistance

### 4. Discussion

To date, the control of hospital-acquired infections caused by multiply resistant gram-negative bacilli is a particular problem. Multi drug resistant *Acinetobacter baumannii* has emerged as a substantial clinical problem worldwide (16,17). *Pseudomonas aeruginosa* is an opportunistic pathogen due to its ability to activate useful phenotypes under environmental stress and to persist in adverse conditions such as antibiotic or antiseptic substances. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are important causes of infections in humans and early identification of infections due to these organisms are important and may reduce mortality rate in burn units.

By a resistant rate of 82.7%, we found gentamycin as the most effective agent against *Pseudomonas aeruginosa* isolates and by a resistant rate of 33%, we found tetracycline as the most effective agent against *Acinetobacter baumannii* isolates.

Although cephalosporins have been considered in patients with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections, many isolates resistant to these antibiotics have been reported in the recent years.

The high rate of resistance of our isolates of *pseudomonas aeruginosa* and *Acinetobacter baumannii* to antibiotics is worrisome.

The frequency of bla CTX-M gene and bla TEM gene in *pseudomonas aeruginosa* were 51% and 19.1%, respectively, while studies from Brazil in 2012 found bla CTX-M in 19.6% of isolates (18) and a previous study in 2009 in Brazil reported bla CTX-M in 4.6% of isolates (19). Our study showed prevalence of *bla TEM* higher than a prior research in Iran (20).

The results obtained for prevalence of *bla CTX-M* and *bla TEM* genes in *Acinetobacter baumannii* were 85% and 12.5%, respectively.

Celenza et al observed *bla TEM* and *bla CTX-M* in 26.1% and 30.4% of *Acinetobacter baumannii* isolates, respectively (21). Numerous studies have investigated the prevalence of *bla TEM* gene among *Acinetobacter baumannii* isolates with varying results (22,23). This is a report about high prevalence of *bla CTX-M* in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in Iran that could explain one of the most important possible reasons for the presence of antibiotic resistance to cephalosporins in this region.

In conclusion, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the main sources of infection in one burn hospital in Tehran, Iran and should be supervised by strict isolation methods to reduce their severe outcomes and mortality rate in burn patients. CTX-M- producing isolates were encountered frequently in this hospital and high prevalence of this gene indicates a considerable risk for its spread among patients.

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