



## Multi-Drug Resistant *Acinetobacter*-Derived Cephalosporinase and OXAsetC Genes in Clinical Specimens of *Acinetobacter* spp. Isolated From Teaching Hospital

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### ABSTRACT

**Background:** Hospital-acquired infections caused by multi-drug resistant *Acinetobacter* spp. are often extremely difficult to treat and this has proved to be a serious problem worldwide.

**Objectives:** The aim of this study was to determine the incidence rates and distribution patterns of multi-drug resistant (MDR) *Acinetobacter* spp. strains and the occurrence of *Acinetobacter*-derived cephalosporinase (ADC-7) and OXA-type carbapenemases (OXAsetC genes) in clinical specimens in the Beheshti Teaching Hospital in Kashan, Iran.

**Materials and Methods:** This descriptive study was carried out on sixty isolates of *Acinetobacter* spp. and clinical samples collected from patients. The level of antibiotic resistance was determined by the disc diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) procedure. Polymerase chain reaction (PCR) amplification of the genetic determinants of resistance was also determined.

**Results:** The resistance rates were; amikacin (80%), tobramycin (68.3%), ceftazidime (60%), ciprofloxacin (55%), piperacillin/tazobactam (51.7%), doxycycline (50%), SXT/TMP (sulfamethoxazole / trimethoprim) (48.3%), levofloxacin (43.3%), gentamicin (40%), imipenem (25%), and sulbactam/ampicillin (20%). The frequency of MDR *Acinetobacter* spp. strains isolated was found to be 56.7%. These isolates were most sensitive to imipenem followed by ampicillin/sulbactam and gentamicin. The prevalence of genes for ADC-7 and OXAsetC in the *Acinetobacter* spp. was; 34 (56.7%) and 32 (53.3%), respectively. The positive percentages of MDR isolates for ADC-7 were 82.4% and for OXAsetC they were 73.5%.

**Conclusions:** Our phenotypic analysis demonstrated that *Acinetobacter* spp. isolates were resistant to most clinically significant antibiotic classes. This is the first report concerning *Acinetobacter*-derived cephalosporinase, blaADC, enzymes in *Acinetobacter* spp. isolates from Iran.

**Keywords:** *Acinetobacter* spp.; *Acinetobacter*-Derived Cephalosporinase; OXA-Type Carbapenemases; Antibiotic Susceptibility; Iran

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The emergence and rapid spread of multidrug-resistant (MDR) isolates of *Acinetobacter* spp. causing nosocomial infections are of great concern worldwide.

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## 1. Background

*Acinetobacter* spp. have emerged as important opportunistic pathogens in hospitals and these are often related to international nosocomial outbreaks (1). These infections are complicated to treat due to the occurrence of multidrug-resistant (MDR) organisms, which include resistance to; beta-lactams, aminoglycosides, fluoroquinolones and more recently, carbapenems (2). There has been increasing reports of carbapenem resistance in *Acinetobacter* strains worldwide (1). Increases in the prevalence of resistant strains has also been seen, which has compromised patient treatment with; aminoglycosides, penicillins, extended spectrum cephalosporins, and more recently, fluoroquinolones (1-3).

The principal mechanism of carbapenem resistance is enzymatic hydrolysis mediated by carbapenemases. In *A. baumannii* these enzymes are usually OXA-type carbapenemases, which are members of class D according to the Ambler classification system (4). The OXA-type carbapenemases are divided into five sub-groups, four of these are acquired carbapenemases and their nomination is according to the circulation of genes *bla*OXA in different geographic areas. Five main phylogenetic subgroups of OXA-type carbapenemases have been recognized in *A. baumannii*; OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like, and OXA-143-like (4).

The major subgroup is OXA-51-like, which is responsible for chromosomal encoded enzymes, thus the natural existence of OXA-type carbapenemases in *A. baumannii* is known worldwide (4). Here, we report an analysis of the antibiotic susceptibility profile and genetic determinants of antibiotic resistance in 60 isolates of *Acinetobacter* spp. obtained from patients in our teaching hospital.

## 2. Objectives

The aims of the present study were to determine the frequency of MDR and the occurrence of ADC-7 and OXAsetC genes of *Acinetobacter* spp. from patients hospitalized in a tertiary teaching hospital.

## 3. Materials and Methods

A total of 60 *Acinetobacter* spp. clinical isolates were collected during January 2010 to September 2010 from the Beheshti Hospital in Kashan, Iran. Conventional biochemical tests such as; glucose, xylose, lactose, indole, ONPG

(nitrophenyl-beta-D-galactopyranoside), fermentation of manitol and maltose, motility and production of H<sub>2</sub>S, were used for the identification of isolated *Acinetobacter* spp. at the species level in 60 Gram negative, short rods showing both a negative reaction on oxidase testing and the lack of lactose fermentation. *A. baumannii* is saccharolytic, and *A. lwoffii* is asaccharolytic. The strains were isolated from; blood (58.4%), sputum (16.7%), urine (13.3%), cerebrospinal fluids (8.3%), and pleural fluid (3.3%) samples.

Antimicrobial susceptibility testing was performed on all 60 isolates according to the standard method established by the CLSIs (5). Imipenem (10µg), ciprofloxacin (5µg), levofloxacin (5µg), ceftazidime (30µg), sulbactam/ampicillin (10/10µg), tazobactam/piperacillin (100/10µg), amikacin (30µg), gentamicin (10µg), tobramycin (10µg), SXT/TMP (1.25/23.75µg), doxycycline (30µg), and minocycline (30µg) disks (Becton Cockeysville, USA, Microbiology Systems ) were used. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls in each susceptibility test. Multidrug resistance was defined in this analysis as resistance to three or more representatives of the following classes of antibiotics; quinolones (ciprofloxacin, levofloxacin), broad-spectrum cephalosporins (ceftazidime), beta-lactamase inhibitor/beta-lactams (sulbactam/ampicillin, tazobactam/piperacillin), aminoglycosides (amikacin, gentamicin and tobramycin), tetracyclines (doxycycline, minocycline), trimethoprim-sulfamethoxazole and carbapenems (imipenem).

All objective genes and related primers used for polymerase chain reaction (PCR) amplification are listed in Table 1. The primers used for these tests were purchased from the Cinagene Company, Iran. For PCR, a 1:10 dilution of 24 hours culture was boiled for 10 min., then amplification was performed with 1:10 of this dilution as the DNA template. PCR conditions included 30 cycles of amplification under the following conditions: Denaturation at 95° C for 30 sec., annealing at 50° C for 30 sec., and then at 40° C for 40 sec., cycling was followed by a final extension at 72° C for 30 min. PCR products were set on 2.0% agarose gels, stained with ethidium bromide, and photo-graphed by UV illumination (Ingenius, Syngene). The 100-bp DNA ladder (Bioneer, Korea) was used to assess the PCR product size. The antimicrobial resistance rates of the isolates were compared using a chi-square test or Fisher's exact test with SPSS version 16 for Windows (SPSS, Chicago, IL).

**Table 1.** Primers for Amplification of Genes From *Acinetobacter* spp. Isolates.

Primer Name	Primer Sequence (5' to 3')	Annealing Temperature (° C)	Target Gene(s)	Reference
ADC-7 FOR	ATGCGATTAAAAAATTCCTGT	50	<i>bla</i> ADC-1, <i>bla</i> ADC-2, <i>bla</i> ADC-3, <i>bla</i> ADC-4, <i>bla</i> ADC-5, <i>bla</i> ADC-6, <i>bla</i> ADC-7	(6)
ADC-7 REV	TTATTTCTTTATTGCATTACG			
OXA SET C FOR	ACAGAARTATTTAAGTGGG	47	<i>bla</i> OXA-51, <i>bla</i> OXA-58, <i>bla</i> OXA-64, <i>bla</i> OXA-69, <i>bla</i> OXA-70, <i>bla</i> OXA-71, <i>bla</i> OXA-75, <i>bla</i> OXA-78	(7)
OXA SET C REV	GGTCTACAKCCMWTCCECA			

#### 4. Results

*Acinetobacter* spp. isolates were recovered from 60 patients including, 35 men (58.3%) and 25 women (41.7%). From 60 *Acinetobacter* spp. isolates in the present study, 48 (80%) were recognized as *A. baumannii*, 10% as *A. lwoffii* and 10% as other genomic species of *Acinetobacter*. The characteristics of the patients are summarized in Table 2.

**Table 2.** Demographic Characteristics of the Study Population.

Parameters	Patients, No. (%) <sup>a,b</sup>
Age, y <40	29 (48.3)
Age, y > 40	31 (51.7)
Sex (male/female)	35/25 (58.3/41.7)
<b>Hospital Sampling Ward</b>	
Emergency room	24 (40)
Internal medicine	15 (25)
ICU	15 (25)
Pediatrics	6 (10)

<sup>a</sup> range[minimum, maximum]: 81[4,85]

<sup>b</sup> Mean  $\pm$  SD: 39.27  $\pm$  19.20

The mean age for the studied population was 39.3 years ( $\pm$  19.2), with a range of 4 to 85 years. Table 3 summarizes the isolation sites and antibiotic resistance patterns in this study. The resistance pattern of *Acinetobacter* spp. isolates in our hospital showed a very high resistance rate to amikacin (80%) and tobramycin (68.3%). Isolated *Acinetobacter* spp. were more sensitive to ampicillin/sulbactam than to the other antibiotics tested. From the 60 isolates, 34 (56.7%) were resistant to at least three classes of antibiotics and classified as multi-drug resistant (MDR). Surprisingly, among the MDR isolates 97.1% were resistant to amikacin, 91.2% to ceftazidime, 85.3% to piperacillin/tazobactam, 82.4% to ciprofloxacin, 73.5% to levofloxacin and trimethoprim/sulfamethoxazole, 70.6% to doxycycline and gentamicin, 67.6% to minocycline, 58.8% to tobramycin, 44.1% to imipenem, and 35.3% to ampicillin/sulbactam.

From the hospital departments and type of specimens the following levels of MDR were found; 83.3% (5/6) of the isolates were from pediatrics, and 80% (12/15) were from ICU, 90% (9/10) from sputum samples and 40% (14/35) of the blood isolates were MDR. The percentage of antibiotic resistance genes detected in *Acinetobacter* spp. strains according to the hospital locations are shown in Table 4. The frequency rates for ADC-7 and OXAsetC in ICU isolates were higher than that found in the other wards. The percentage rates of antibiotic sensitivity and resistance patterns of *Acinetobacter* spp. isolates in relation to the genes encoding beta-lactamases detected, are shown in Ta-

ble 5. More than 80% of the OXA-type carbapenemase positive isolates were resistant to amikacin. The highest resistance rate of *ampC* *Acinetobacter*-derived cephalosporinase positive isolates were observed in ceftazidime and amikacin, respectively. The *ampC* *Acinetobacter*-derived cephalosporinase, *blaADC*, was detected in 57.6% of the stains by PCR amplification. Thirty strains from a total of 34 (88.2%) *blaADC* positive strains were resistant to ceftazidime and 28 out of 34 (82.4%) were MDR. By using PCR, a *blaOXA-69*-like gene was found in 53.3% of the isolates, and 25 out of 32 strains (78.1%) were resistant to ceftazidime and MDR, simultaneously.

#### 5. Discussion

*Acinetobacter* spp. has emerged as a major pathogen of nosocomial infections, while the management of this pathogen, due to the increased emergence of carbapenem-resistant strains, has become a significant challenge (3). Its low level susceptibility to ceftazidime (40%) is very important. This may be the consequence of an enhanced production of class-C chromosomal beta-lactamase (*AmpC*), which is possibly related to the extensive use of cephalosporin antibiotics in our hospital. Overall, piperacillin/tazobactam with a 48.3% susceptibility rate is the best treatment choice. Imipenem with a 75% susceptibility rate, exhibited the best in vitro antimicrobial action compared to the other beta-lactamases. Susceptibility to imipenem varies around the world. Previous reports indicated approximately; 51% imipenem-sensitive isolates in Iran (8), 43.4% to 56.7% in Turkey (9), and 10% to 55% in Saudi Arabia (8). Imipenem-resistant strains were analyzed for their genetic determinants. Sixty percent of them were positive for *blaOXA-51*-like gene and 80% for ADC-7 genes.

It has been shown that the majority of the carbapenem resistance in *A. baumannii* is due to the production of carbapenemases, especially those belonging to the carbapenem-hydrolyzing class D beta-lactamases, which are encoded by the *blaOXA-51*-like, *blaOXA-23*-like, *blaOXA-24*-like, and *blaOXA-58*-like genes (3). The *blaOXA-51*-like gene is native and was originally located on the chromosome of *A. baumannii*. It is chromosomally positioned and is extensively prevalent. Similar to other class of D enzymes, they have a better attraction for imipenem than meropenem. Their role in carbapenem resistance is related to the presence of an insertion sequence ISAb1, situated upstream, possibly providing a promoter for the hyperproduction of beta-lactamase genes (3, 10, 11). A wide diversity of OXA genes exist among the strains of *A. baumannii* isolated in Iran (8). A high incidence of OXA-type carbapenemase in MDR *A. baumannii* has been reported in the northwest of Iran (12).

**Table 3.** Frequency of Antibiotic Resistance Patterns of 60 *Acinetobacter* spp. Isolates in Relation to Various Specimens From Hospitalized Patients

	Ciprofloxacin	Levofloxacin	Ceftazidime	Tobramycin	Amikacin	Gentamicin	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	Piperacillin-tazobactam	Apicillin-sulbactam,	Minocycline	Doxycycline	MDR
Blood stream	15	9	16	25	27	8	12	8	12	5	14	17	14
Cerebrospinal fluid	3	4	3	4	5	3	4	1	3	3	3	3	3
Urine	5	4	5	6	5	4	6	4	5	2	5	6	6
Pleural fluid	2	1	2	1	2	1	1	0	2	0	1	1	2
Sputum	8	8	10	5	9	8	6	2	9	2	6	3	9
Total	33	26	36	41	48	24	29	15	31	12	29	30	34

**Table 4.** Percentage Rate of Antibiotic Resistant Genes Detected in Sixty Isolated *Acinetobacter* spp. According to Hospital Location.

Genes Encoding b-Lactamases	Gene Detection in Isolates, No. (%)	ICU	Emergency Room	Pediatrics	Internal Medicine
ADC-7	34 (56.7)	13 (38.2)	6 (17.6)	4 (11.8)	11 (32.4)
OXAset C <sup>a</sup>	32 (53.3)	12 (37.5)	8 (25)	4 (12.5)	8 (25)

<sup>a</sup> OXAsetC: includes *blaOXA-51*, *blaOXA-58*, *blaOXA-64*, *blaOXA-69*, *blaOXA-70*, *blaOXA-71*, *blaOXA-75* and *blaOXA-78*-like genes.

**Table 5.** Percentage Rates of Antibiotic Sensitivity and Resistance Patterns Detected in Sixty *Acinetobacter* spp. Isolates in Relation to Genes Encoding β-Lactamases.

	OXA Set C Positive			ADC-7 Positive		
	S	I	R	S	I	R
Amikacin (30μg)	9.3	9.4	81.3	8.8	5.9	85.3
Gentamicin (10μg)	31.2	0	68.8	29.4	0	70.6
Tobramycin (10μg)	15.6	37.5	46.9	14.7	35.3	50
Tazobactam/ Piperacillin (100/10μg)	18.8	6.2	75	14.7	8.8	76.5
Sulbactam/Ampicillin (10/10μg)	46.9	28.1	25	28.2	29.4	32.4
Ceftazidime (30μg)	12.5	9.4	78.1	5.9	5.9	88.2
Ciprofloxacin (5μg)	6.2	21.9	71.9	11.8	14.7	73.5
Levofloxacin (5μg)	28.1	0	71.9	29.4	0	70.6
Imipenem (10μg)	68.8	3.1	28.1	61.8	2.9	35.3
Doxycycline (30μg)	12.5	34.4	53.1	8.8	29.4	61.8
Minocycline (30μg)	9.4	25	65.6	8.8	20.6	70.6
SXI/TMP (1.25/23.75μg)	12.5	25	62.5	12.5	25	62.5

Abbreviations: S, sensitive; I, intermediate; R, resistance

Chromosomal-encoded *blaOXA* genes play a challenging role in the antibiotic resistance of *A. baumannii* (13). The *blaOXA-51*-like genes are omnipresent in *A. baumannii*. Insertion of ISAbal upstream from *blaOXA-51*-like provides a strong promoter, resulting in enhanced gene expression and carbapenem resistance (14, 15). Chromo-

somally encoded *Acinetobacter*-derived cephalosporinases (ADCs) are intrinsic in all *A. baumannii* strains. The presence of ISAbal elements are highly associated with increased *AmpC* gene expression and resistance to extended-spectrum cephalosporins (16). More than 60% of ADC-7 gene positive isolates were sensitive to imipen-

em. Cefepime and carbapenems were shown to be stable in response to these enzymes (6). In *Acinetobacter* spp., beta-lactamases are the most common factors creating resistance to imipenem and extended-spectrum cephalosporins, including extended-spectrum beta-lactamases (ESBL), carbapenem-hydrolyzing class D beta-lactamases (CHDL), *Acinetobacter*-derived cephalosporinases (ADC), and metallo-beta-lactamases (MBL) (17). Mobile genetic elements, may contribute to beta-lactamase overproduction and distribution (17).

The identification of imperative OXA-type carbapenemases and *Acinetobacter*-derived cephalosporinases in this study confirms the wide distribution of these enzymes in our hospital. An increase in the numbers of these enzymes may cause extended intra- and inter-hospital spread; therefore, it is necessary to design an effective protocol in order to control infections caused by *A. baumannii* which are resistant to carbapenems.

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## Authors' Contribution

None declared.

## Ethical Consideration

All ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc.) have been carefully considered.

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