

The First Report of CMY, aac (6´)-Ib and 16S rRNA Methylase Genes Among *Pseudomonas aeruginosa* Isolates From Iran

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ABSTRACT

Background: Serious infections by *Pseudomonas aeruginosa* are commonly treated with the combination of a beta-lactam antibacterial and an aminoglycoside. Therefore, production of a 16S rRNA methylase may result in an extremely important antibacterial resistance profile.

Objectives: The present study was conducted to determine the prevalence of Cephamycinase (CMY), aminoglycoside 6´-N-acetyltransferase (aac(6´)-Ib) and 16S rRNA methylase genes among *Pseudomonas aeruginosa* isolates from Iran.

Patients and Materials: This descriptive study was performed on hospitalized burnt patients during 2011 and 2012. Antibiotics susceptibility tests were performed by disc diffusion and broth microdilution methods. CMY, aac(6´)-Ib, 16S rRNA methylase genes were detected by PCR method.

Results: Seventy-seven (77%) of 100 isolates were resistant to Imipenem and Ceftazidime. aac(6)-Ib, Cephamycinase (CMY), and rRNA methyltransferase (rmtB and rmtD) were detected in 57 (74.02%), 7 (9.09%), 11 (14.28%), and 9 (11.68%) isolates, respectively. PCR results for aminoglycoside resistance methyltransferase (armA) and rRNA methyltransferase (rmtC) were all negative. Aminoglycoside resistance methyltransferase (armA), and rRNA methyltransferase (rmtC) were not detected.

Conclusions: This study detected multiple drug resistance in *Pseudomonas aeruginosa* including resistance to β -lactams, Aminoglycosides, and Fluoroquinolones. Therefore, identification of drug resistance patterns in *P. aeruginosa* and detection of pan-resistant producing isolates are of great importance in prevention and control of infections in burn center ward.

Keywords: *Pseudomonas aeruginosa*; Drug Resistance, Multiple, Bacterial; Burn Units

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► Implication for health policy/practice/research/medical education:

The present study was conducted to determine the prevalence of CMY, aac(6´)-Ib-cr and 16S rRNA methylase genes among Metallo- β -Lactamase-producing *Pseudomonas aeruginosa* isolates from Iran.

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1. Background

Treatment of infections caused by *Pseudomonas aeruginosa* is becoming increasingly complicated by its tendency to acquire resistance to multiple classes of antibacterials. The agents commonly used to treat these infections include Antipseudomonal, Penicillins, Aminoglycosides, Carbapenems, Fluoroquinolones, and Cephalosporins (1). The emergence of resistance to antibiotics in *P.aeruginosa* is a serious concern. The beta-lactamases-producers are capable of hydrolyzing almost all β -lactam antibiotics and are associated with high morbidity and mortality (2). Methylation of 16S rRNA has emerged as a mechanism of high-level Aminoglycoside resistance among bacteria in recent years, and prevention of the adverse effects of intrinsic Aminoglycosides that would block their own 16S rRNA. Aminoglycosides continue to play an important role in the management of serious infections caused by bacteria, often in combination with broad-spectrum beta-lactams. Since 2003, the production of 16S rRNA methylase has been detected to be a novel mechanism of aminoglycoside resistance. So far, seven types of methylases have been identified (ArmA, RmtA, RmtC, RmtB, RmtE, RmtD, and NpmA). Also, these genes are mediated by bacterium-specific recombination systems, such as transposons, and are easily translocated to other DNA target sites (3). AmpC beta-lactamases hydrolyze expanded-spectrum cephalosporins. The most plasmid-mediated AmpC β -lactamases belong to the DHA, FOX, and CMY families (4). In 1989, Bauernfeind et al. detected a *K. pneumoniae* isolate from South Korea that could transfer resistance to cefoxitin and cefotetan as well as to Penicillins, Oxymino-cephalosporins, and Monobactams to *E.coli*. The enzyme, termed CMY-1 for its Cephamycinase activity, and was more sensitive to be inhibited by Sulbactam than Clavulanate or Tazobactam, suggesting that it might be a class C enzyme (5). Since 1998, three plasmid-mediated quinolone resistance (PMQR) mechanisms have been described, namely Qnr, *aac(6)-Ib-cr* and QepA. The rise in multiresistant bacteria harboring *aac(6)-Ib* has seriously limited the effectiveness of Amikacin and other Aminoglycosides. These mechanisms are widely prevalent amongst common clinical isolates (6). The present study was conducted to determine the prevalence of these enzymes among *P. aeruginosa* clinical isolates from Iran.

2. Objectives

The present study was conducted to determine the prevalence of CMY, *aac(6)-Ib* and 16S rRNA methylase genes among *Pseudomonas aeruginosa* isolates from Iran.

3. Patients and Methods

3.1. Isolation and Clinical Identification

From September to January 2011, 448 burnt patients referred to Shahid Motahari hospital (level I burn care center) in Tehran, Iran were recruited. 100 isolates of *P. aeruginosa* were collected by sterile swabs; before ulcers were washed by physiological serum. Samples were transferred in stuart media, cultured on Cetrimide and MacConkey agar and incubated at 37°C for 24 h. Colonies that made pigment or odor, were studied using biochemical tests such as oxidase test, catalase, sugar fermentation test, and ability of growth in 42°C. The isolates were stored at -200c in brain heart broth containing 20% glycerol. *Pseudomonas aeruginosa* ATCC27853 was used as a control strain.

3.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility to Imipenem (IPM, 10 μ g), Meropenem (MEM, 10 μ g), Ceftazidime (CAZ, 30 μ g), Amikacin (AK, 30 μ g), Tobramycin (TOB, 10 μ g), Piperacillin/Tazobactam (PTZ, 100/10 μ g), Ciprofloxacin (CIP, 5 μ g), Cefepime (FEP, 30 μ g), Ceftriaxone (CRO, 30 μ g), Aztreonam (ATM, 30 μ g), Gentamicin (GEN, 10 μ g) and Carbenicillin (Car, 100 μ g) (Mast Group, Merseyside, UK) was performed by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Merck, Germany) based on clinical laboratory standards institute (CLSI) guidelines (7). *Pseudomonas aeruginosa* ATCC27853 was used as a control strain.

3.3. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of different antibiotics (Imipenem, Meropenem, Cefepime, Ampicillin, Piperacillin/Tazobactam, Ceftriaxone and Ceftazidime (GLAXO England Co. and Himedia Co.) were determined according to the guidelines of the CLSI by broth microdilution method (7).

3.4. Detection of Resistance Genes

DNA templates were prepared by boiling method. All clinical *P.aeruginosa* isolates were screened for the presence of CMY, *aac(6)-Ib*, *armA*, *rmtB*, *rmtC* and *rmtD* by polymerase chain reaction (PCR) amplification in a thermal cycler (Eppendorf, Master cycler gradient). The primer pair sequences used in the study has been shown in Table 1, and genes were amplified under the following condition.

3.5. Nucleotide Sequence Accession Number

The nucleotide sequence data reported in this paper have been submitted to the GenBank sequence database

and assigned accession no. JX648311 and JX644173.

Table 1. Oligonucleotide Primers Used in This Study

Nucleotide Sequence	Primer	Target	PCR Conditions			Size (bp)
			Denaturing	Anneal	Extension	
(5'-ATGATGAAAAATCGTTATG-3') (5'-TTGTAGCTTTCAAGAATGC-3')	CMY-F, CMY-R	CMY	94°C, 45 s	55°C, 45 s	72°C, 45 s	635
(5'-GCTTCTCGGGCGATGTA-3') (5'-ATGCAATGCCGCGCTCGTAT-3')	rmtB-F, rmtB-R	rmtB	94°C, 45 s	55°C, 45 s	72°C, 45 s	173
(5'-CGAAGAAGTAACAGCCAAAG-3') (5'-ATCCCAACATCTCTCCACT-3')	RmtC-F, RmtC-R	rmtC	94°C, 45 s	55°C, 45 s	72°C, 45 s	711
(5'-ATTCTGCCTATCTAATTGG-3') (5'-ACCTATACITTTATCGTCGTC-3')	ArmA-F, ArmA-R	armA	94°C, 45 s	55°C, 45 s	72°C, 45 s	315
(5'-CGGCACGCGATTGGGAAGC-3') (5'-CGGAAACGATGCGACGAT-3')	RmtD-F, RmtD-R	rmtD	94°C, 30 s	55°C, 30 s	72°C, 30 s	401
(5'-TTGCGATGCTCTATGAGTGGCTA-3') (5'-CTCGAATGCTGGCGTGTTT-3')	<i>aac(6)-Ib</i> -F, <i>aac(6)-Ib</i> -R	<i>aac(6)-Ib</i>	94°C, 45 s	55°C, 45 s	72°C, 45 s	482

4. Results

All *P. aeruginosa* isolates were resistant to Meropenem, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime, and Carbenicillin. Seventy-seven (77%) of 100 isolates were resistant to Imipenem and Ceftazidime, while 49% of isolates were resistant to Gentamicin. Also isolates were multidrug resistant (MDR) (resistant to more than three antibiotics from different classes were defined as MDR). *aac(6)-Ib*, CMY, *rmtB* and *rmtD* were detected in 57 (74.02%), 7 (9.09%), 11 (14.28%), and 9 (11.68%) isolates, respectively. No *armA* and *rmtC* genes were detected in isolates (Figure 1).

5. Discussion

Recent 20 years, *P. aeruginosa* is known as the most common bacteria in burn wards in Tehran, Iran (8). 16S rRNA methylases can confer high-level resistance to aminoglycosides (3). These genes have been found in *Pseudomonas aeruginosa* and other bacteria in many areas. The dissemination mechanisms of 16S rRNA methylases genes are of great clinical importance. In the present study, the *rmtB* gene was more prevalent than the *armA*, *rmtD* and *rmtC* genes in *P. aeruginosa* isolates. The *rmtB* and *rmtD* genes were found in 11 (14.28%) and 9 (11.68%) isolates, respectively and *armA* and *rmtC* were not found totally. A number of different pan-aminoglycosides resistance-promoting 16S rRNA methylases have been detected in *P. aeruginosa*, including *RmtA*, *RmtB*, *RmtD* and *ArmA* (9). In a study from China, *armA* or *rmtB* were detected in 94.7% (89/94) of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains, and *armA* was predominant (84 vs. 5 strains with *rmtB*). No *rmtA*, *rmtC*, *rmtD* or *npmA* genes were found (10). In a study from France, four *armA* isolates with positive results were detected in 2005 (n = 2) and in

2007 (n = 2), in addition to a single isolate with positive result from 2006. No isolate had positive results for the

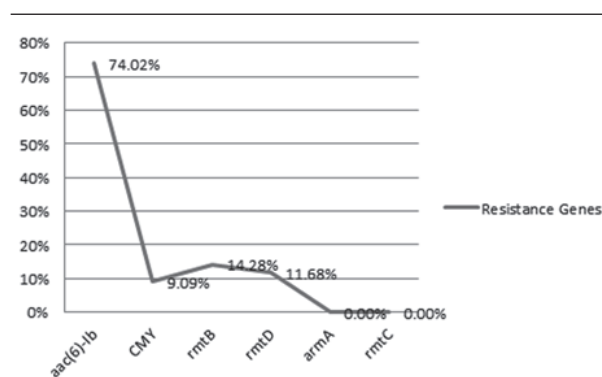


Figure 1. The prevalence of Resistant Genes in *P. aeruginosa* Isolates From Patients in Burn Ward

rmtA, *rmtC*, *rmtD*, and *npmA* genes (11). The *amp-C* beta-lactamases constitute a group of enzymes widely spread in gram-negative bacteria which inactivate Cephalosporins such as Ceftazidime and Cefotaxime (4). The CMY gene was detected in 7 (9.09%) of isolates. In Taiwan, five isolates were found to produce CMY-2 AmpC enzymes in a total of 1210 isolates, and one isolate carried both CTX-M-3 and CMY-2, also three and nine isolates expressed putative AmpC β -lactamases (12). A novel aminoglycoside acetyltransferase that exhibits FQ-acetylating, *aac(6)-Ib-cr*, has been detected in *P. aeruginosa* (9). The *aac(6)-Ib* gene was detected in 57 (74.02%) of isolates. In a study from the United States, among 313 *Enterobacteriaceae*, *aac(6)-Ib* was present in 50.5% of isolates (13). The prevalence of coproduction of *rmtB*, *rmtD*, *aac(6)-Ib* and CMY in this collection of

Imipenem-resistant *P. aeruginosa* isolates was 3 (3.8%). This is the first report of CMY, aac(6)-Ib and 16S rRNA methylase genes among *Pseudomonas aeruginosa* isolates from Iran. Therefore, control and treatment of these infections caused by the mentioned bacteria is difficult, so there is a need for revised treatment protocols to prevent resistant genes dissemination among clinical isolates.

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Authors' Contributions

None declared.

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