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Short Communication

Prevalence of resistance to aminoglycosides and fluoroquinolones among *Pseudomonas aeruginosa* strains in a University Hospital in Northeastern Poland

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Abstract

The present study was conducted to investigate the prevalence of genes encoding resistance to aminoglycosides and fluoroquinolones among twenty-five *Pseudomonas aeruginosa* isolated between 2002 and 2009. In PCR, following genes were detected: *ant(2'')-Ia* in 9 (36.0%), *aac(6')-Ib* in 7 (28.0%), *qnrB* in 5 (20.0%), *aph(3'')-Ib* in 2 (8.0%) of isolates.

Key words: *Pseudomonas aeruginosa*, plasmid-mediated resistance to aminoglycosides and fluoroquinolones, aminoglycoside-modifying enzymes.

Pseudomonas aeruginosa is a non-fermentative, Gram-negative bacterium widespread in the natural and artificial environment. Characteristic feature of this pathogen is a remarkable ability to develop antimicrobial resistance, thus infections caused by multidrug-resistant (MDR) strains are associated with high mortality rate and elevated treatment cost (Lister *et al.*, 2009). Many studies report that selection of highly resistant mutants occurs in Intensive Care Units and *P. aeruginosa* is a main cause of nosocomial infections (Wolska *et al.*, 2012). Resistance to antibiotics may be linked both with chromosomal mutations and acquisition of resistance genes located on mobile genetic elements, such as plasmids, integrons, and transposons (Lister *et al.*, 2009). From variety of plasmid-mediated aminoglycoside resistance mechanisms, the most commonly encountered is the production of aminoglycoside-modifying enzymes (Tada *et al.*, 2013). High level of resistance to aminoglycosides can also be mediated with production of 16S rRNA methyltransferases, which preclude disturbance of protein synthesis caused by aminoglycoside molecule (Doi and Arakawa, 2007). Currently ten genes encoding these enzymes were detected, of which the most common are *armA* and *rmtB* (Deng *et al.*, 2013). Plasmid-associated resistance to fluroquinolones can be mediated by the production of Qnr proteins, which preserve DNA gyrase and topoisomerase IV from inhibition by quinolones (Poirel, 2012). This mechanism contributes to low-level fluoroquinolone resistance, but it is able to broadening the mutant selecting window (Drlica and Zhao, 2007).

The aim of this study was to determine the prevalence of plasmid-mediated genes encoding aminoglycoside-modifying enzymes, 16S rRNA methyltransferases, and Qnr-like proteins among MDR *P. aeruginosa* strains.

Twenty-five nonduplicated *P. aeruginosa* strains were obtained from patients hospitalized in two Intensive Care Units at University Hospital of Bialystok (northeastern Poland) between July 2002 and October 2009. Isolates were selected due to their reduced susceptibility to aminoglycosides, fluoroquinolones, third- and fourth generation cephalosporins, and/or carbapenems. Identification and susceptibility testing were conducted using an automated VITEK 2 system with AST-N093 cards (bioMérieux, Marcy l'Etoile, France). Susceptibility to antibiotics was interpreted according to the EUCAST criteria published on February 11, 2013 (The European Committee on Antimicrobial Susceptibility Testing, 2013). The minimal inhibitory concentrations (MICs) of gentamicin, amikacin, netilmicin, ciprofloxacin, imipenem, meropenem, ceftazidime, and cefepime were determined by Etest technique (bioMérieux). Plasmid material was isolated from over-

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Table 1 - Specific primers used for assays and conditions of each PCR reaction.

Target	Primer	Nucleotide sequence	PCR conditions					Size (bp)	Source of primers sequence
			Predenaturation	Denaturation	Annealing	Elongation	Final elongation		
aac(3)-Ia	aac3-F aac3-R	5'GGCTCAAGTATGGGCATCAT 5'TCACCGTAATCTGCTTGCAC	94 °C, 5 min[a]	94 °C, 45 s, 30x	52 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	389	This study
aac(6')-Ib	aacA4-F aacA4-R	5'GCTCTTGGAAGCGGGGACGG 5'TCGCTCGAATGCCTGGCGTG	94 °C, 5 min[a]	94 °C, 45 s, 30x	55 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	300	Sacha *et al.*
ant(4')-IIa	ant4pr-F ant4pr-R	5'ATCGTCTGCGAGAAGCGTAT 5'TAAAACGCCTATCCGTCACC	94 °C, 5 min[a]	94 °C, 45 s, 30x	52 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	839	This study
ant(2'')-Ia	ant2bi-F ant2bi-R	5'GACACAACGCAGGTCACATT 5'CGCAAGACCTCAACCTTTTC	94 °C, 5 min[a]	94 °C, 45 s, 30x	55 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	500	This study
aph(3'')-Ib	aph3bi-F aph3bi-R	5'CTTGGTGATAACGGCAATTCC 5'CCAATCGCAGATAGAAGGCAA	94 °C, 5 min[a]	94 °C, 45 s, 30x	52 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	548	Madsen 2000
armA	armA-F armA-R	5'TATGGGGGTCTTACTATTCTGCCTAT 5'TCTTCCATTCCCTTCTCCTTT	94 °C, 5 min[a]	94 °C, 45 s, 30x	54 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	514	Fritsche 2008
rmtB	rmtB-F rmtB-R	5'TCAACGATGCCCTCACCTC 5'GCAGGGCAAAGGTAAAATCC	94 °C, 5 min[a]	94 °C, 45 s, 30x	54 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	459	Fritsche 2008
qnrA	qnrA-F qnrA-R	5'ATTTCTCACGCCAGGATTTG 5'GATCGGCAAAGGTTAGGTCA	94 °C, 5 min[b]	94 °C, 45 s, 32x	53 °C, 45s, 32x	72 °C, 60 s, 32x	72 °C, 7 min	516	Robicsek 2006
qnrB	qnrB-F qnrB-R	5'GATCGTGAAAGCCAGAAAGG 5'ACGATGCCTGGTAGTTGTCC	94 °C, 5 min[b]	94 °C, 45 s, 32x	54 °C, 45s, 32x	72 °C, 60 s, 32x	72 °C, 7 min	463	Robicsek 2006
qnrS	qnrS-F qnrS-R	5'ACGACATTCGTCAACTGCAA 5'TAAATTGGCACCCTGTAGGC	94 °C, 5 min[b]	94 °C, 45 s, 32x	54 °C, 45s, 32x	72 °C, 60 s, 32x	72 °C, 7 min	417	Robicsek 2006
ampC	ampC-F ampC-R	5'CGCATACCAGATTCCCCTG 5'CATGTCGCCGACCTTGTAGT	94 °C, 5 min[a]	94 °C, 45 s, 30x	54 °C, 45s, 30x	72 °C, 60 s, 30x	72 °C, 10 min	873	This study

[a]PCR conditions were designed for this study.
[b]PCR conditions were adapted from Robicsek 2006.

night cultures by Plasmid Mini Kit (A&A Biotechnology, Gdynia, Poland). Screening of *ampC* gene was performed by polymerase chain reaction (PCR) with specific primer pair. Primers for amplification of *aac(6')-Ib*, *aac(3)-Ia, ant(4')-IIa, ant(2")-Ia, aph(3")-Ib, armA, rmtB, qnrA, qnrB, qnrS* genes were designed from sequences deposited in the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/) or were selected from the literature (Table 1). Conditions of each PCR reaction are listed in Table 1. All PCR assays were performed in the LabCycler Gradient (SensoQuest GmbH, Goettingen, Germany). Sequencing of genes encoding aminoglycoside-modifying enzymes was conducted with primers listed in Table 1, using the 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA).

The genes encoding aminoglycoside-modifying enzymes were identified in plasmid material of 13 strains (52.0%). PCR assays revealed the presence of *ant(2")-Ia* gene in nine (36.0%), *aac(6')-Ib* gene in seven (28.0%), and *aph(3")-Ib* gene in two (8.0%) strains. Three isolates harbored two genes encoding aminoglycoside-modifying enzymes: *aac(6')-Ib* and *ant(2")-Ia* in two strains; *ant(2")-Ia* and *aph(3")-Ib* in one strain. One isolate carried three genes for resistance to aminoglycosides: *aac(6')-Ib*, *ant(2")-Ia*, and *aph(3")-Ib*. *QnrB* gene related with plasmid-mediated resistance to quinolones was detected in five (20.0%) strains. Sequencing of the PCR-positive products confirmed the presence of *ant(2")-Ia*, *aac(6')-Ib*, *aph(3")-Ib*, and *qnrB1* genes in particular strains (GenBank accession numbers: *ant(2")-Ia* X04555.1; *aac(6')-Ib* JF901756.1; *aph(3")-Ib* M28829.1, *qnrB1* DQ777878.1). Genes *aac(3)-Ia*, *ant(4')-IIa*, *armA*, *rmtB*, *qnrA*, and *qnrS* were not identified in plasmid DNA of tested strains. Characteristic of MDR strains with identified genes for resistance to aminoglycosides and quinolones are shown in Table 2. The highest efficiency among antimicrobials showed ceftazidime (68.0% of all tested strains were susceptible). The only aminoglycoside active against tested strains was amikacin (8.0% of all tested strains). Higher resistance rates were observed in strains carrying genes encoding aminoglycoside-modifying enzymes, than in strains without this genes detected. Level of resistance to ciprofloxacin was noticeably higher in strains harboring *qnrB* gene than in strains without this gene identified (MIC_{50}: ≥ 32 *vs.* MIC50: 8). As for carbapenems, more isolates were susceptible to imipenem (28.0%) than meropenem (24.0%).

Over the years, numerous studies reported the increasing prevalence of MDR *P. aeruginosa* in hospital environments all around the world. The present study focused on the investigation of plasmid-mediated resistance to aminoglycosides and fluoroquinolones in hospital located in northeastern Poland. The most frequently detected gene was *ant(2")-Ia* (36.0%). Spanish research also revealed that *ant(2")-Ia* gene occurs most often among

Table 2 - Characteristics of MDR *P. aeruginosa* strains with identified genes encoding aminoglycoside-modifying enzymes and Qnr-like proteins.

Isolate	Specimen	Year of isolation	Genotype	MIC (μg/mL)						
				GM	AN	NC	CIP	IMP	MEM	FEP	CAZ
PS-05	urine	2003	*aac(6')-Ib+ant(2")-Ia +qnrB*	≥ 256	64	≥ 256	≥ 32	≥ 32	16	48	4
PS-07	bronchial secretion	2003	*qnrB*	8	64	≥ 256	≥ 32	16	2	32	2
PS-09	bronchoalveolar lavage	2003	*aac(6')-Ib*	16	128	≥ 256	4	2	2	16	4
PS-10	urine	2004	*aac(6')-Ib +qnrB*	32	128	≥ 256	≥ 32	16	2	4	2
PS-12	bronchial secretion	2004	*ant(2")-Ia*	≥ 256	64	32	4	2	16	16	2
PS-15	nasal swab	2005	*aac(6')-Ib*	64	≥ 256	≥ 256	≥ 32	16	≥ 32	32	32
PS-16	bronchial secretion	2005	*ant(2")-Ia*	≥ 256	64	≥ 256	1	≥ 32	16	16	8
PS-17	bronchoalveolar lavage	2006	*aac(6')-Ib +qnrB*	≥ 256	≥ 256	≥ 256	≥ 32	≥ 32	≥ 32	16	64
PS-18	blood	2006	*ant(2")-Ia*	≥ 256	128	≥ 256	8	16	1	48	32
PS-19	bronchial secretion	2007	*ant(2")-Ia*	≥ 256	≥ 256	≥ 256	≥ 32	≥ 32	≥ 32	4	1
PS-21	bronchial secretion	2008	*ant(2")-Ia*	≥ 256	≥ 256	≥ 256	≥ 32	16	≥ 32	32	64
PS-22	bronchial secretion	2008	*aac(6')-Ib+ant(2")-Ia+aph(3")-Ib*	16	128	≥ 256	≥ 32	≥ 32	16	48	16
PS-23	nasal swab	2009	*aac(6')Ib+ant(2")-Ia*	128	≥ 256	≥ 256	≥ 32	≥ 32	2	32	2
PS-25	bronchial secretion	2009	*ant(2")-Ia+aph(3")-Ib +qnrB*	≥ 256	128	8	≥ 32	≥ 32	≥ 32	32	1

GN = gentamicin; AN = amikacin; NC = netilmicin; CIP = ciprofloxacin; IMP = imipenem; MEM = meropenem; FEP = cefepime; CAZ = ceftazidime.

P. aeruginosa strains – it was identified in 65.0% (Fernandez *et al.*, 2013), while in Iranian study it was observed in 28.0% of tested isolates (Vaziri *et al.*, 2011). Our earlier investigation conducted on MDR *P. aeruginosa* reported the presence of *aac(6')-Ib* gene in 58.3% of isolates (Sacha *et al.*, 2012), whereas in this assay it was detected in 28.0% of tested strains. PCR study performed to detect genes involved in the production of Qnr-like proteins revealed the presence of *qnrB* in 20.0% of tested strains. Among *Enterobacteriaceae* screened for production of plasmid-mediated fluoroquinolone resistance determinants, *qnrB* was reported as most prevalent gene (Kim *et al.*, 2009). Earlier Polish study demonstrated that aminoglycoside and fluoroquinolone resistance rates were comparable to our results: amikacin (91.0% *vs.* 92.0%), gentamicin (98.0% *vs.* 100.0%), ciprofloxacin (98.0% *vs.* 100.0%). Percentage of strains resistant to beta-lactams was even higher: 93% were resistant to ceftazidime, 89% to cefepime, 41% to imipenem, 88% to meropenem (Paluchowska *et al.*, 2012). Resistance rates of MDR isolates obtained from 10 Spanish hospitals were similar to those of our strains in the case of imipenem (66.67% *vs.* 72%), ceftazidime (40% *vs.* 32%), cefepime (73.33% *vs.* 88%) (Cabot *et al.*, 2012).

This research focused on investigating the most commonly reported plasmid-mediated factors of aminoglycoside and fluoroquinolone resistance, and further assays are necessary to determine the other causes of antimicrobial resistance.

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