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SHORT COMMUNICATION

VIA MEDICA

Occurrence of the *aacA4* gene among multidrug resistant strains of *Pseudomonas aeruginosa* isolated from bronchial secretions obtained from the Intensive Therapy Unit at University Hospital in Bialystok, Poland

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Abstract: The aim of this study was to investigate the prevalence of the *aacA4* gene in a population of multidrug resistant strains of *P. aeruginosa* isolated from bronchial secretions obtained from the Intensive Therapy Unit (ITU). Twelve MDR isolates were tested for antibiotic susceptibility and the presence of the *aacA4* gene. In this study, 58.3% of the strains contained (6')-Ib' aminoglycoside acetyltransferase gene. All of the studied strains (*aacA4-positive* and *aacA4-negative*) were susceptible only to colistine (100%). Among other antibiotics, the lowest resistance rates were those shown against ceftazidime (14.3% to 20%) and imipenem (28.6% to 40%). Our studies frequently revealed the presence of the *aacA4* gene as a factor responsible for resistance; it is probable that other mechanisms of resistance to aminoglycoside antibiotics also occurred. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 2, 322–324)*

Key words: *Pseudomonas aeruginosa*, *aacA4* gene, antimicrobial susceptibility

Introduction

Pseudomonas aeruginosa is a leading Gram-negative bacterial pathogen associated with nosocomial infections. It is responsible for a wide range of clinical manifestations, including pneumonia, urinary tract infection, and bacteremia [1].

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In intensive therapy unit (ITU) patients, it may behave as an opportunistic pathogen, causing severe invasive diseases, and represents one of the most severe nosocomial pathogens [2]. The spread of this organism is often difficult to control, due to the presence of many intrisinic and acquired mechanisms of antimicrobial resistance [3, 4].

Aminoglycosides with β-lactams are an important component of antipseudomonal therapy. The inactivation of these drugs by modifying enzymes is the commonest mechanism of aminoglycoside resistance [5].

The aim of the present study was to investigate the prevalence of the resistance-modifying enzyme gene, *aacA4 (*synonym: *aac(6')-Ib' or aac(6')-4),* in multidrug resistant strains of *P. aeruginosa* isolated

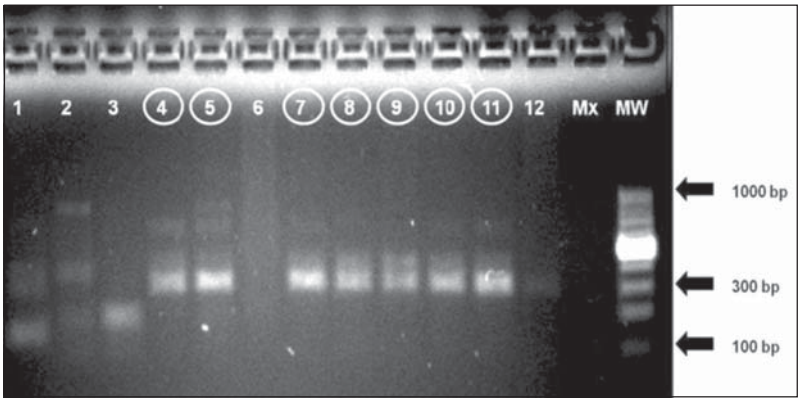

Figure 1. Detection of the aacA4 gene by the use of electrophoresis in agarose gel. Lanes: MW, molecular marker (Perfect™ 100–1,000 bp DNA ladder; EURx, Poland); Lanes: 4, 5, 7, 8, 9, 10, 11, *P. aeruginosa aacA4-positive* strains; 1, 2, 3, 6, 12, *P. aeruginosa aacA4-negative* strains; Mx, negative control (no DNA added)

from bronchial secretions obtained from the Intensive Therapy Unit at University Hospital in Bialystok, Poland.

Material and methods

Bacteria. A total of 12 multidrug resistant *Pseudomonas aeruginosa* strains were included in this study. All strains were isolated from bronchial secretions received from the Intensive Therapy Unit and identified using the automated VITEK 2 System (bioMérieux, France). *P. aeruginosa* (ATCC 27853) served as a control strain antimicrobial susceptibility test and as a negative control in PCR experiments.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by using AST-N093 cards (bioMérieux) and the automated VITEK 2 System. MICs were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (available at: http://www.eucast.org/clinicalbreakpoints/).

PCR amplification of *aacA4* gene. Plasmid DNA of *P. aeruginosa* strains was extracted using a Plasmid Mini Kit (A&A Biotechnology, Poland) according to the manufacturer's instructions.

The *aacA4* gene was detected with a specific primer pair: forward, JG-aacA4-F: 5'-GCTCTTGGAAGCGGGGACGG-3' and reverse, JG-aacA4-F: 5'-TCGCTCGAATGCCTGGCGTG-3'. The PCR mixtures contained: 12.5 μl PCR RED Master Mix (DNA-Gdansk II, Poland), 1 μl of each primer, 3 μl of template plasmid DNA and 7.5 μl of ultra pure H_2O to final 25 μl volumes. These primers were used to amplify a 300 bp fragment of *aacA4* gene.

PCR was performed in a LabCycler Gradient (SensoQuest GmbH, Germany) thermal cycler. Amplification was carried out as follows: initial denaturation for 5 min at 94°C, 35 cycles of 30 s at 94°C, 45 s at 94°C and 20 s at 72°C, and a final elongation step for 10 min at 72°C. Amplification products were visualized after running at 5V/cm for 60 min in 1.5% agarose gel containing 0.5 μg/ml ethidium bromide (BioRad, USA). A Perfect™ 100–1,000 bp DNA ladder (EURx, Poland) was used as a size marker.

Results and discussion

PCR analysis revealed the presence of resistance gene *aacA4*, which encoded aminoglycoside 6'-N-acetyltransferase (AAC(6')-Ib C), in seven out of 12 (58.3%) multidrug resistant strains of *P. aeruginosa* (Figure 1).

In our study, all examined strains of *P. aeruginosa* were resistant to aminoglycoside antibiotics (gentamicin, amikacin and tobramycin) and antipseudomonal penicillins (piperacillin, ticarcillin and ticarcillin/clavulanic acid) except colistine (100% susceptible). The results presented here indicate that the *aacA4* gene may affect aminoglycoside resistance, although probably other mechanisms also participate in this type of resistance.

The resistance rates to other antibiotics (base on MICs) in the group of *aacA4*-positive strains were as follows: cefepime — 57.1%, ceftazidime — 14.3%, imipenem — 28.6%, meropenem and ciprofloxacin — 85.7% (Table 1). The observed resistance rates to the above antibiotic were similar in the *aacA4*-negative group (from 20% ceftazidime to 80% ciprofloxacin). The strains of *P. aeruginosa* were significantly resistant to meropenem. Similar findings were reported by Strateva et al. [6] in strains from ITU — a rate of resistance to meropenem of 61.4% and to ciprofloxacin of 80.3%.

The occurence of *aacA4* gene in the majority of tested strains showed that the dominant mechanism

Table 1. MIC results and aminoglycoside susceptibility profiles among *aacA4-positive* and *aacA4-negative* strains of *P.aeruginosa* isolated from bronchial secretions (from the Intensive Therapy Unit; ITU)

Antibiotics	MIC [mg/L]													
	aacA4-positive								*aacA4-negative*					
	4 Psa	5 Psa	7 Psa	8 Psa	9 Psa	10 Psa	11 Psa	R %	1 Psa	2 Psa	3 Psa	6 Psa	12 Psa	R %
Gentamycin	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	100%	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	100%
Amikacin	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	100%	≥ 64	32	≥ 64	≥ 64	≥ 64	100%
Tobramycin	8	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	≥ 16	100%	≥ 16	8	≥ 16	≥ 16	≥ 16	100%
Cefepime	16	16	16	8	16	8	4	57.1%	8	16	8	8	≥ 64	40%
Ceftazidime	8	8	8	8	16	8	8	14.3%	4	8	4	8	≥ 64	20%
Imipenem	≥ 16	≥ 16	≤ 1	4	8	8	8	28.6%	≤ 1	≥ 16	≤ 1	4	≥ 16	40%
Meropenem	≥ 16	≥ 16	1	≥ 16	≥ 16	≥ 16	≥ 16	85.7%	1	≥ 16	1	≥ 16	≥ 16	60%
Piperacillin	64	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	64	100%	64	≥ 128	64	≥ 128	≥ 128	100%
Ticarcillin	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	100%	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	100%
Ticarcillin/ -clavulanic acid	≥ 128	≥ 128	≥ 128	64	≥ 128	64	32	100%	64	≥ 128	64	64	≥ 128	100%
Ciprofloxacin	1	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4	85.7%	1	≥ 4	≥ 4	≥ 4	≥ 4	80%
Colistine	2	2	2	2	2	2	2	0%	2	2	2	2	2	0%

of resistance to aminoglycosides was ability to produce aminoglycoside 6'-N-acetyltransferase. Highly plausible is the presence of other mechanisms resistance to aminoglycoside antibiotics, both in the population of strains *aacA4*-positive and *aacA4*-negative [7].

In conclusion, although aminoglycosides remain useful antipseudomonal agents, resistance to these drugs continues to be a major issue, especially in the therapy of multidrug resistant strains of *Pseudomonas aeruginosa* isolated from ITU patients. Because these aminoglycoside resistance genes are usually located on mobile genetic elements like plasmid, transposon, or integrons [8], there is growing concern that they could easily spread and be disseminated among other bacteria such as *Staphylococcus aureus* [9] or *Enterobacteriacae* [10, 11].

The design of novel aminoglycosides with a stronger affinity for their targets and resistance to these modifying enzymes is inevitable, and the new generation of anti-*Pseudomonas* therapy is forthcoming [12].

Aminoglycoside resistance among clinical strains of *P. aeruginosa* promises to become a major clinical concern, and continuous local surveillance of aminoglycoside resistance is crucial.

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