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

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\textbf{Abstract:} The aim of this study was to investigate the prevalence of the \textit{aacA4} gene in a population of multidrug resistant strains of \textit{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 \textit{aacA4} gene. In this study, 58.3\% of the strains contained (6')-Ib' aminoglycoside acetyltransferase gene. All of the studied strains (\textit{aacA4-positive} and \textit{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 \textit{aacA4} gene as a factor responsible for resistance; it is probable that other mechanisms of resistance to aminoglycoside antibiotics also occurred. (\textit{Folia Histochemica et Cytobiologica} 2012, Vol. 50, No. 2, 322–324)

\textbf{Key words:} \textit{Pseudomonas aeruginosa}, \textit{aacA4} gene, antimicrobial susceptibility

\textbf{Introduction}

\textit{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].

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 intrinsic 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, \textit{aacA4} (synonym: \textit{aac(6')-Ib'} or \textit{aac(6')-4}), in multidrug resistant strains of \textit{P. aeruginosa} isolated
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’TGGCTCGAAATGGCCTGCGGTG-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 H2O 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 occurrence of *aacA4* gene in the majority of tested strains showed that the dominant mechanism

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**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)
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 Enterobacteriaceae [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.

Acknowledgments

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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)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC [mg/L]</th>
<th>aacA4-positive</th>
<th>aacA4-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 Psa</td>
<td>5 Psa</td>
<td>7 Psa</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥ 64</td>
<td>≥ 64</td>
<td>≥ 64</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>8</td>
<td>≥ 16</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Cefepime</td>
<td>8</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥ 16</td>
<td>≥ 16</td>
<td>1</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>128</td>
<td>≥ 128</td>
<td>≥ 128</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>128</td>
<td>≥ 128</td>
<td>≥ 128</td>
</tr>
<tr>
<td>Ticarcillin/ -clavulanic acid</td>
<td>128</td>
<td>≥ 128</td>
<td>≥ 128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>≥ 4</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Colistine</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

References


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