Identification of plasmid OXA and other \(\beta\)-lactamase genes among carbapenem-resistant isolates of *Pseudomonas aeruginosa* from a clinical university hospital in North Eastern Poland

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SUMMARY

The aim of the study was to evaluate the prevalence of OXA and other \(\beta\)-lactamase genes, antibiotic susceptibility, and the genetic relatedness among clinical isolates of *P. aeruginosa* resistant to carbapenems. The presence of blaOXA genes was demonstrated in 48% of isolates belonging to four PFGE profiles. Most of them contained the blaOXA-2 gene (88.3%). Other blaOXA genes (Ps1310 with blaOXA-30 and Ps1309 with blaOXA-10) were found in only two isolates. The tested isolates also contained other \(\beta\)-lactamase genes such as blaVIM-2, blaVIM-4, blaSHV-1, and blaTEM-1. All isolates were susceptible only to colistin (100%).

KEY WORDS: *P. aeruginosa*, blaOXA genes, Antibiotic susceptibility, PFGE.
tests (AST-N259 cards) were performed using the VITEK 2 automated system (bioMérieux, USA). The results of susceptibility to antibiotics were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf).

Plasmid DNA extractions from *P. aeruginosa* isolates were performed with the Plasmid Mini Kit (A&A Biotechnology, Poland) according to the manufacturer’s instructions. Genomic Mini AX Bacteria Kit (A&A Biotechnology, Poland) was used for the isolation of total DNA. Polymerase chain reaction (PCR) for *blaOXA* genes (Aktas et al., 2008; Lin et al., 2012) and genes encoding other β-lactamases (*blaKPC, blaVIM, blaIMP, blaSHV, blaTEM, blaCTX-M*) were performed using specific primers and conditions as described previously (Sundsfjord et al., 2004; Ellington et al., 2007; Sacha et al., 2012; Ojdana et al., 2014). Amplicons were sequenced in an external laboratory (Genomed S.A., Warsaw, Poland) and the obtained sequences were compared by using the Basic Local Alignment Search Tool (BLAST) database http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&amp;PAGE_TYPE=BlastSearch&amp;LINK_LOC=blasthome.

Clonal relationships among all tested *P. aeruginosa* isolates were determined by pulsed-field gel electrophoresis (PFGE) of genomic DNA. PFGE typing was performed according to a previously described procedure (Yetkin et al., 2006) with some modifications. Isolates were grown for 16 h at 37°C in tryptic soy broth (TSB) (Oxoid, UK). The cultures were centrifuged and the resulting pellet suspended in 1 mL of TEN buffer (pH 8.0; 0.1 M Tris, 0.1 M ethylenediaminetetraacetic [EDTA], 0.15 M NaCl). Next, the suspension was centrifuged again and re-suspended in 100 µL of EC buffer (pH 7.5; 6 mM Tris, 1M NaCl, 0.1 M EDTA, 0.5% [w/v] Brij 58, 0.2% [w/v] sodium deoxycholate, 0.5% sodium lauroyl sarcosinate), and cells were embedded into 2% low-gelling-temperature agarose (Type VII; Sigma-Aldrich, USA). After overnight digestion with 50 mg/mL of lysozyme (Sigma-Aldrich) and then 1 mg/mL of

![Dendrogram of carbapenem resistant *P. aeruginosa* isolates.](image-url)
protease (Sigma-Aldrich), genomic DNA in the agarose plugs was restricted by 20 U of XbaI (EurX, Poland) for 6 h at 37°C. DNA fragments were resolved in 1.2% agarose gel (Pulsed Field Certified Agarose, BIO-RAD, USA) with 0.5 × Tris-borate-EDTA (TBE) buffer at 6 V/cm at 14°C using a CHEF Mapper XA Chiller System (BIO-RAD). Pulse times were 4–35 s for 20 h. The gel was stained with ethidium bromide (Sigma-Aldrich) (5 µg/mL), and photographed under UV light using a ChemiDoc XR System (BIO-RAD). NTSYS pc 2.02 (Exeter Software, USA) and the unweighted pair group method with arithmetic mean algorithm (UPGMA) was used to prepare a dendrogram of genetic relatedness between tested isolates. Band position tolerance was set on 1.5%.

The presence of blaOXA genes was demonstrated

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Breakpoints (mg/l) of isolates with blaOXA genes</th>
</tr>
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<tbody>
<tr>
<td>Ps1309 1</td>
<td>≥64 16 16 ≥4 ≤0.5 ≥16 ≤0.5 4 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1310 2</td>
<td>≥64 ≥64 ≤1 0.5 ≤0.5 8 ≥16 4 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1311 *1</td>
<td>8 16 ≥4 ≤0.5 ≥16 ≥16 16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1314 *2</td>
<td>8 16 ≥4 ≤0.5 ≥16 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1315 *3</td>
<td>8 16 ≥64 ≥4 ≤0.5 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1318 *3</td>
<td>8 16 ≥4 2 ≥16 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1319 *3</td>
<td>8 16 ≥4 2 ≥16 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1320 *3</td>
<td>8 16 ≥4 2 ≥16 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1321 *4</td>
<td>8 16 ≥64 ≥4 2 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1322 *4</td>
<td>8 16 ≥64 ≥4 2 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1323 *3</td>
<td>8 16 ≥64 ≥4 2 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1325 *3</td>
<td>8 16 ≥64 ≥4 2 ≥16 ≥16 ≥16 ≥16</td>
</tr>
</tbody>
</table>

TABLE 1 - Comparison of antibiotic susceptibility of P. aeruginosa isolates with and without blaOXA genes.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Breakpoints (mg/l) of isolates without blaOXA genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps1201</td>
<td>8 8 4 ≥4 ≤0.5 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1203</td>
<td>16 32 16 1 1 8 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1204</td>
<td>16 16 16 ≥4 ≤0.5 8 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1205</td>
<td>≤2 8 8 1 ≤0.5 ≤1 8 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1206</td>
<td>≤2 ≥64 ≥64 ≥4 2 4 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1300</td>
<td>≤2 ≤1 2 ≥4 2 ≤1 ≥16 ≤1 4 ≤1</td>
</tr>
<tr>
<td>Ps1306</td>
<td>≤2 8 8 0.5 2 ≤1 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1311 *6</td>
<td>264 16 ≥64 ≥4 2 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1312 7</td>
<td>≤2 8 4 0.5 1 ≤1 ≥16 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1316 *8</td>
<td>264 8 16 ≥4 ≤0.5 ≥16 ≥16 ≥16 ≥16</td>
</tr>
<tr>
<td>Ps1317</td>
<td>≤2 8 8 ≤0.25 2 ≥16 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1318</td>
<td>4 2 4 ≤0.25 2 ≥16 4 ≥16 ≤1</td>
</tr>
<tr>
<td>Ps1323 *6</td>
<td>264 ≥64 ≥64 ≥4 ≤0.5 ≥16 ≥16 ≥16 ≥16</td>
</tr>
</tbody>
</table>

| % S | 57.1% 57.1% 50% 28.6% 100% 50% - - 71.4% |
| % I | 14.3% - - 14.3% - - 14.3% 21.4% |
| % R | 28.6% 42.9% 50% 57.1% - 50% 85.7% 78.6% 28.6% |

AN, amikacin; FEP, cefepime; CAZ, ceftazidime; CIP, ciprofloxacin; CS, colistin; GM, gentamicin; IPM, imipenem; MEM, meropenem; TM, tobramycin; S, susceptible; I, intermediate; R, resistant; *1, isolates with blaOXA-2 and blaOXA-10; *2, isolates with blaOXA-30; *3, isolates with blaVIM-2; *4, isolates with blaVIM-2 and blaVIM-4; *5, isolates with blaVIM-1; *6, isolates with blaVIM-1; *7, isolates with blaVIM-1; *8, isolates with blaVIM-1.
in 48% (12/25) of the tested isolates (Figure 1). Most of the blaOXA-positive isolates contained the blaOXA-2 gene (88.3%). Only two isolates contained other blaOXA genes (Ps1310 isolate with blaOXA-10 and Ps1309 with blaOXA-10).

Molecular typing of 25 non-repetitive P. aeruginosa isolates identified 13 PFGE profiles. Presented results indicated that seven (63.6%) of the isolates with blaOXA genes belonged to one large profile (G2), and were isolated from August to December 2013 from ICU patients. Only two strains, Ps1309 (G1) and Ps1310 (B), isolated at a different time (only in May 2013), contained other genes.

Plasmid-encoded VIM β-lactamases were identified in five P. aeruginosa isolates (Ps1311, Ps1321, Ps1322, Ps1323 with blaVIM-2, and Ps1202 with blaVIM-4). Moreover, two (Ps1321 and Ps1322) of the five isolates contained blaOXA-2 genes. Other β-lactamase genes were observed in only two isolates (Ps1312 with TEM-1 and Ps1316 with SHV-5).

The previously observed higher frequency of class D β-lactamases versus class A indicates the geographical distribution of these genes (Lee et al., 2005). The most common enzyme was OXA-10 (40.8%), followed by OXA-1 (22.5%) and OXA-2 (20.4%). The blaOXA-10 gene is the most frequently encountered gene in P. aeruginosa (Yan et al., 2006; Heintz et al., 2010; Vatcheva-Dobrevska et al., 2013). Also in our study, the majority of detected β-lactamases belonged to group D in comparison to groups B and A (44%, 20%, and 8%, respectively). In contrast to the above-cited studies, OXA-2 was the most prevalent in carbapenem-resistant P. aeruginosa isolates (Ps1202 with blaOXA-10 and Ps1309 with blaOXA-10).

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REFERENCES


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