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Regional Spread of *Pseudomonas aeruginosa* ST357 Producing IMP-7 Metallo-β-Lactamase in Central Europe[▽]

Metallo-β-lactamases (MBLs) hydrolyze penicillins, cephalosporins, and carbapenems. Since the mid-1990s, organisms with acquired MBLs, mainly *Pseudomonas aeruginosa* and, more recently, various members of the *Enterobacteriaceae*, have become a significant epidemiologic problem worldwide (15, 16). The spread of MBL producers in Poland commenced in the late 1990s (13, 19) and has been continuing to date, with predominance of *P. aeruginosa* with VIM-type MBLs (5, 14; M. Gniadkowski, unpublished data). In September 2006, the first and so far the only *P. aeruginosa* isolate with an IMP-like enzyme was identified within the ongoing national surveillance of MBL producers by the National Medicines Institute in Warsaw (out of all 44 MBL-producing *P. aeruginosa* strains from 22 centers in 2006). It was recovered from a patient in a hospital in Wrocław, in the southwestern part of Poland. In contrast, the first two MBL-producing *P. aeruginosa* isolates in the Czech Republic were identified in 2008. They were recovered in a hospital in Ústí nad Labem (north of the country) and found to produce the IMP-7 enzyme (8). Together with another IMP-producing isolate from 2009 from a hospital in Prague, these were three of all eight MBL-producing *P. aeruginosa* from four centers, collected in 2008–2009 during the national surveillance of antimicrobial resistance by the National Reference Laboratory for Antibiotics in Prague. The aim of this study was to compare the four IMP producers from the transborder region of the two countries.

The phenotypic MBL detection in all of the isolates was performed by the double-disk synergy test with disks containing imipenem, ceftazidime, and EDTA (11) and was verified by the spectrophotometric assay for imipenem hydrolysis in the presence and absence of EDTA (1). MICs of 12 antimicrobials were determined by broth microdilution and interpreted according to the methods of the CLSI (2). The isolates showed identical MIC patterns, with *in vitro* susceptibility to piperacillin, amikacin, and colistin only (Table 1). The PCR identification of bla_{IMP} genes was carried out with primers Imp-F and Imp-R (4), followed by amplification and sequencing of entire genes (8). Similarly to the isolates from Ústi nad Labem (8), the isolates from Prague and Wrocław carried the bla_{IMP-7} gene. The PCR and sequencing methodology proposed previously (5) allowed localization of the gene inside class 1 integrons and characterization of their variable regions. The regions were amplified in two parts with primers specific for bla_{IMP} genes (4) and integronic conserved segments, 5′-CS and 3′-CS (5); the 5′ parts were amplified with primers 5CS and Imp-R, whereas the 3′ parts were amplified with primers Imp-F and 3CS. The entire regions were sequenced and consisted of three gene cassettes, *aac(6′)-Ib*–bla_{IMP-7}–*aac(3)-I*, which is a unique structure. A mating experiment was performed with all the isolates and the recipient rifampin-resistant *P. aeruginosa* PAO1161 as described before (5); no transconjugants were obtained on plates with 8 μg/ml imipenem. Total DNA from the isolates was purified in agarose plugs as described previously (18), and undigested DNA and DNA digested with S1 nuclease (Takara, Otsu, Japan) were run by pulsed-field gel electrophoresis (PFGE). No DNA bands that could be assigned to plasmids were observed. Late-logarithmic cultures of the isolates were subjected to a plasmid purification procedure with the Qiagen plasmid midi-kit (Qiagen, Hilden, Germany). The preparations obtained were treated with EcoRI (Fermentas, Vilnius, Lithuania) and electrophoresed along with the isolates' total DNAs cut with the same restriction enzyme. No banding patterns were visualized in gel lanes with "plasmid" preparations. The PFGE and conventional gels were blotted onto a Hybond-N+ membrane (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) and hybridized with a probe, the amplicon specific for the bla_{IMP-7} gene (4). The experiments were performed with the ECL random-prime labeling and detection system (Amersham Pharmacia Biotech). The only hybridization signals were obtained with the genomic DNAs and not with any cryptic bands that might be produced by a plasmid(s) (data not shown). These results suggested the chromosomal location of the bla_{IMP-7} and lack of plasmids in the isolates. The isolates were typed by PFGE (18) and by multilocus sequence typing (MLST) using the procedure and the database available at http://pubmlst.org (3, 9). All of the isolates had the same PFGE pattern and belonged to sequence type ST357.

This study showed the emergence of a rare variant of multidrug-resistant organism and its spread in a limited area so far. According to the MLST database (http://pubmlst.org), *P. aeruginosa* ST357 has been reported only in Singapore in 2008 to date. Although prevalent in Asia, in Europe IMP-producing *P. aeruginosa* has been observed mostly in Italy so far (17). IMP-7 has been reported in Canada (6), Malaysia (7), and Japan (10, 20), but to our knowledge no MLST of the producer *P. aeruginosa* strains has been performed. Integronic arrays with bla_{IMP-7} in the Canadian and Japanese isolates were different than those here (6, 20). Interestingly, clonal *P. aeruginosa* IMP-7 isolates were identified in 2006 in Slovakia, and despite the lack of the integron and MLST data (12), these might have represented the same clone variant as in this work. This would indicate a wider spread of the ST357 IMP-7 clone in Central Europe; however, data from the Czech and Polish surveillance programs show that organisms with VIM-like enzymes predominate in both countries (J.

TABLE 1. Antimicrobial susceptibility of the IMP-7-producing *P. aeruginosa* isolates ($n = 4$)

MIC (μg/ml)[a]											
PIP	TZP	CAZ	CFP	FEP	ATM	IPM	MEM	AMK	GEN	CIP	CST
16	32	>16	>32	>32	16	>16	>16	8	16	>16	1

[a] Abbreviations: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CFP, cefoperazone; CIP, ciprofloxacin; CST, colistin; FEP, cefepime; GEN, gentamicin; IPM, imipenem; MEM, meropenem; PIP, piperacillin; TZP, piperacillin-tazobactam.

Hrabák and H. Žemličková, unpublished data; M. Gniadkowski, unpublished data).

Nucleotide sequence accession number. The gene cassette structure *aac(6′)-Ib–bla*$_{IMP-7}$*–aac(3)-I* was deposited in GenBank under accession no. HM021184.

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