Nosocomial Outbreak of Carbapenem-Resistant *Pseudomonas aeruginosa* with a New *bla*$_{IMP}$ Allele, *bla*$_{IMP-7}$

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Pseudomonas aeruginosa **isolates from an outbreak in Canada were highly resistant to carbapenems and ceftazidime but not piperacillin. They produced a novel integron-associated metallo-β-lactamase, designated IMP-7, with 91% identity to IMP-1.** ***bla*$_{IMP-7}$** **was not detected with standard** ***bla*$_{IMP}$**-specific primers, owing to mismatches in the forward primer.**

Multidrug resistance in *Pseudomonas aeruginosa* arises if a strain undergoes multiple mutations, derepressing chromosomal AmpC β-lactamases, up-regulating efflux, and decreasing permeability. Alternatively, *P. aeruginosa* can develop multidrug resistance by acquiring plasmids, transposons, or integrons that encode potent β-lactamases and aminoglycoside-modifying enzymes. Recent interest has centered on the emergence of strains with IMP and VIM metallo-β-lactamases (9). These enzymes hydrolyze most β-lactams, including carbapenems, and are encoded by integrons that often also specify aminoglycoside 6′-*N*-acetyltransferases (6, 11, 12).

Outbreaks of *P. aeruginosa* infection. *P. aeruginosa* isolates resistant to carbapenems, aminoglycosides, ciprofloxacin, and ceftazidime but apparently susceptible to piperacillin were recovered during 1995 and 1996 from nine patients in two rehabilitation wards at the Bow Valley Center at Calgary General Hospital (CGH) in Calgary, Alberta, Canada. Most of the isolates were from cultures of urine from patients with spinal cord injuries. At the end of 1996, the rehabilitation program was moved to the Foothills Hospital (FHH), also in Calgary. From January to October 1997, 15 patients in FHH were identified with nosocomial acquisition of *P. aeruginosa* with a multidrug resistance phenotype similar to that seen at CGH in 1995 and 1999. Most, but not all, of the patients in FHH had direct contact with the rehabilitation or intensive care units. No such *P. aeruginosa* isolates had been encountered at FHH in 1996. Most were again from urine, but there were also isolates from burn wounds, surgical wounds, sacral and perineal ulcers, lower respiratory specimens, and external ear swabs. No isolates were from cultures of blood or specimens from other normally sterile sites. Cultures of environmental specimens did not reveal a source of the organism, and carbapenems were rarely used at either hospital. Isolation and infection control practices were reinforced on several occasions, and the outbreak ceased late in 1997.

Characterization of isolates. Susceptibility was determined by various means (see below): with a Vitek system (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, Mo.), by broth microdilution with Pasco panels (Becton-Dickinson, Wheatridge, Colo.), by the Kirby-Bauer disk methodology, by agar dilution (10), and with the Etest (AB Biodisk Solna, Sweden). Isolates were tested by disk diffusion with ceftazidime in the presence of 2-mercaptopropionic acid to detect metallo-β-lactamases (1). Total DNA was fingerprinted by pulsed-field gel electrophoresis (PFGE) after digestion with *Spe*I (New England Biologicals, Beverly, Mass.) (15). Crude cell extracts were prepared by freeze-thawing and were assayed against 0.1 mM imipenem by spectrophotometry at 297 nm, as described previously (18). Conjugative transfer of carbapenem resistance to *P. aeruginosa* PU21 (5) was attempted by plate mating with selection on agar with rifampin (100 μg/ml) and meropenem (10 μg/ml).

Detection and analysis of metallo-β-lactamase genes. PCR assays for *bla*$_{IMP}$ and *bla*$_{VIM}$ were performed by use of previously published primers and amplification conditions (13, 17). Genomic DNA fragments from a representative isolate (isolate 98/P/6327) were cloned into pBC SK(+) (Stratagene, Cambridge, United Kingdom). Recombinant plasmids were electroporated into *Escherichia coli* XL-1 Blue MRF′ (Stratagene), and transformants likely to contain β-lactamase genes were selected with ampicillin (10 μg/ml), as described previously (18). One recombinant plasmid was used for initial sequencing with primers specific for conserved class I integron sequences (7). The sequence obtained was compared with published sequences by using CLUSTAL W software (16).

Resistance phenotypes. Multiple isolates of *P. aeruginosa* retrieved from CGH in 1995 and 1996 and FHH in 1997 had unusual antibiograms, with resistance to gentamicin, tobramycin, ciprofloxacin, and ceftazidime but with susceptibility to piperacillin, as determined by the criteria of NCCLS (10). Carbapenems were not routinely tested with the Vitek and Pasco panels used in the study. The isolates gave no zones around 10-μg imipenem or meropenem disks, nor did they give zones around those containing cefepime at 30 μg. Meropenem and ceftazidime MICs were both subsequently found to be

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TABLE 1. MICs determined with the Etest for the outbreak strain (three representative isolates), *E. coli* XL-1 Blue MRF′ containing vector pBC SK(+), and *E. coli* XL-1 Blue MRF′ containing vector pBC SK(+) with recombinant phagemid pARL98-3

Antibiotic	MIC (μg/ml)		
	Outbreak strain	pBC SK(+)	pARL98-3 (bla_{IMP-7})
Imipenem	>32	0.25	0.25
Meropenem	>32	0.012	0.125
Ampicillin	—[a]	4	32
Aztreonam	16	0.25	0.125
Cefoxitin	—	16	>256
Cefpirome	—	0.125	1.0
Ceftazidime	>256	0.25	>256
Piperacillin	8–16	1	1
Cefepime	>32	—	—
Piperacillin-tazobactam	8–16	—	—
Tobramycin	64–128	—	—
Amikacin	32	—	—
Ciprofloxacin	>32	—	—

[a] —, not done.

>512 μg/ml by agar dilution. The MICs of all relevant drugs were determined in parallel for a representative group of strains with the Etest, confirming the resistance of the isolates to all drugs except piperacillin and piperacillin in combination with tazobactam (Table 1).

Isolates with this antibiogram from both CGH and FHH showed consistent PFGE banding profiles, indicating clonality; other *P. aeruginosa* isolates from the same period but with different antibiograms exhibited different banding patterns. Attempts to transfer carbapenem resistance to *P. aeruginosa* PU21 were unsuccessful.

Characterization of β-lactamases. The level of resistance to ceftazidime was reduced in the presence of 2-mercaptopropionic acid, suggesting the involvement of a metallo-β-lactamase (1). Crude cell extracts from four representative isolates hydrolyzed 0.1 mM imipenem rapidly in spectrophotometric assays (8), confirming carbapenemase production. Nevertheless, PCR assays for bla_{IMP} and bla_{VIM} were repeatedly negative. Although the bla_{IMP}-specific primers were originally designed to amplify bla_{IMP-1}, they also detect bla_{IMP-2} (12), bla_{IMP-3} (4), and bla_{IMP-4} (3); likewise, the bla_{VIM}-specific primers detect both bla_{VIM-1} (6) and bla_{VIM-2} (11).

On the basis of this failure to detect alleles of known carbapenemase genes, genomic DNA from a representative isolate was cloned. A cell extract prepared from a selected transformant hydrolyzed imipenem, although phenotypic carbapenem resistance was not apparent (Table 1). The recombinant plasmid in this clone contained an insert of >10 kb, and the plasmid was designated pARL98.3. Sequencing of a 3.0-kb segment between the conserved sequences characteristic of

type I integrons revealed four complete gene cassettes (Fig. 1). The first sequence was an unknown open reading frame with all the characteristics of an integron cassette (14): namely, a core site (GTTRRRY), an inverse core site, and a 59-bp element. The second and fourth cassettes contained *aacC4* and *aacC1*, respectively, both of which encode aminoglycoside acetyltransferases. The third cassette, designated bla_{IMP-7}, encoded a class B β-lactamase that shares >86% amino acid identity with known IMP enzymes (Fig. 2). The failure of the original bla_{IMP}-specific primers to amplify bla_{IMP-7} may be explained by four mismatches among 20 bases in the forward primer (data not shown). The reverse primer showed complete homology. Alternative primers specific for bla_{IMP} (1) also have mismatches with bla_{IMP-7} and so would probably not detect the new allele.

Imipenem resistance was observed in 5.2% of 1,466 *P. aeruginosa* isolates during a recent survey in Canada (2). Mechanisms of resistance were not defined, but most of these isolates probably lacked the OprD porin; this mechanism, which arises by a simple point mutation, reduces meropenem susceptibility and confers imipenem resistance. Carbapenemase-mediated imipenem resistance in *P. aeruginosa* is much rarer and has not previously been reported from the Americas. The catalogue of acquired carbapenemases that have been reported is, however, growing sharply (9).

It remains uncertain whether IMP-7 was the sole cause of resistance in the isolates tested in the present study, and permeability lesions may also be needed in order for IMP enzymes to protect bacteria against carbapenems (13). Thus, cloned IMP-7 gave only slight protection against meropenem in *E. coli* XL-1 Blue and no protection against imipenem or aztreonam (Table 1), whereas resistance to ceftazidime and cefoxitin was evident in the transformants.

Microbiologists should be alert to the emergence of an integron-associated carbapenemase gene in *P. aeruginosa* in North America and to the risk that this will spread among regions and species. The need for infection control, which ultimately brought these outbreaks to an end, and for the cautious and prudent use of carbapenems should be underscored. The presence of two aminoglycoside resistance genes in the integron evaluated in the present study implies that it might be selected by these drugs as well as by β-lactams, and it is notable that neither CGH nor FHH used carbapenems extensively. Retention of moderate susceptibility to piperacillin by the isolates examined in the present study is interesting but anomalous, since IMP enzymes hydrolyze the compound, but was observed previously in some IMP-1 producers (13). The clinical efficacy of piperacillin against metallo-β-lactamase producers has not been demonstrated.

Imipenem MICs above 8 to 32 μg/ml should raise the possibility of the presence of a carbapenemase in *P. aeruginosa*,
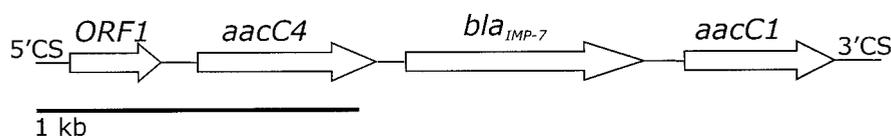

FIG. 1. Orientation of class I integron cassettes in clone pARL98.3. 5′CS and 3′CS, conserved sequences of a class I integron; ORF1, unknown open reading frame; *aacC4*, aminoglycoside resistance gene; bla_{IMP-7}, class B β-lactamase gene; *aacC1*, aminoglycoside resistance gene.

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                       *        20         *        40         *        60
IMP-1   : MSKLSVFFIFLFCSIATAAESLPDLKIEKLDEGVYVHTSFEEVNGWGVVPKHGLVVLVNA
IMP-2   : .K..F.LCVCFL...TA.GAR........E.................S.........T
IMP-3   : ............................................................
IMP-4   : ..................P...................................D.
IMP-7   : .K.....M......ASG.A..................................T


                       *        80         *        100        *        120
IMP-1   : EAYLIDTPFTAKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSRSIPTYASELT
IMP-2   : D.........T......N...........T.................Q.........
IMP-3   : ..............................................G.........
IMP-4   : ..............................................Q.........
IMP-7   : D.............................................Q.........


                       *        140        *        160        *        180
IMP-1   : NELLKKDGKVQATNSFSGVNYWLVKNKIEVFYPGPGHTPDNVVVWLPERKILFGGCFIKP
IMP-2   : ..............K......S.................Q.........K.......V..
IMP-3   : ............................................................
IMP-4   : ...........K...G.........................L.............
IMP-7   : ..............K.....AS.....K...I...............HRV......V..


                       *        200        *        220        *        240
IMP-1   : YGLGNLGDANIEAWPKSAKLLKSKYGKAKLVVPSHSEVGDASLLKLTLEQAVKGLNESKK
IMP-2   : D........L........I.M...V......S....I......R.W.........
IMP-3   : ..............................G.........
IMP-4   : .........L.........I.........A.........
IMP-7   : .........L.........V......................R.........


IMP-1   : PSKPSN
IMP-2   : ..Q...
IMP-3   : ......
IMP-4   : ...L..
IMP-7   : L.....
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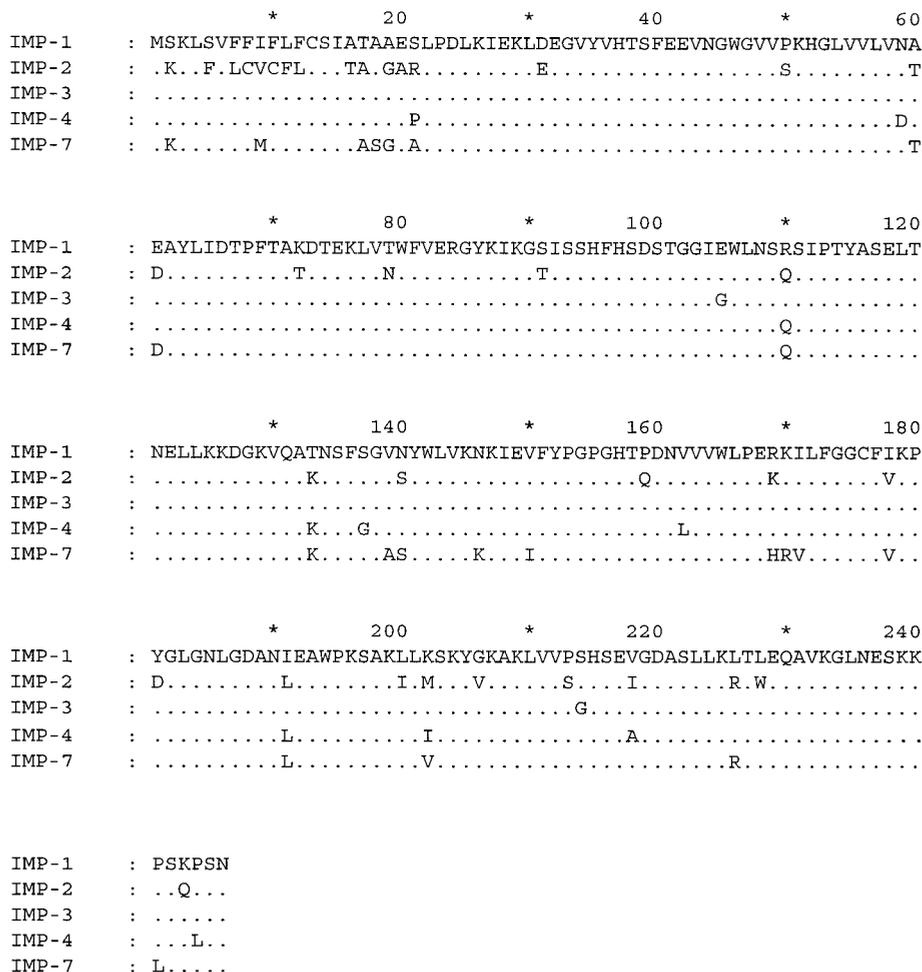
FIG. 2. Sequence of IMP-7 β-lactamase in comparison to those of other published IMP variants.

especially if the isolates are highly resistant to ceftazidime but not, perhaps, piperacillin. PCR has been used to screen for IMP and VIM genes, but as shown here, some bla_{IMP} alleles are sufficiently divergent to preclude detection with standard primer pairs. Tests based on inhibition of metallo-β-lactamase activity by thiol compounds are better screens (1) but use compounds with significant toxicities. Because of this constraint and because some carbapenemases (e.g., SME-1, NMC-A, and OXA-23 to OXA-27) are not metalloenzymes, hydrolysis assays with crude cell extracts are probably the best first step in carbapenemase detection.

Nucleotide sequence accession number. The sequence of the integron obtained in the present study was assigned GenBank accession no. AF318077.

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