Multidrug-Resistant *Pseudomonas aeruginosa* Producing PER-1 Extended-Spectrum Serine-ß-Lactamase and VIM-2 Metallo-ß-Lactamase

To the Editor: In *Pseudomonas aeruginosa*, secondary beta-lactamas with extended substrate specificity can be responsible for acquired resistance to the most powerful antipseudomonal beta-lactams, such as expanded-spectrum cephalosporins and carbapenems (1). A number of these enzymes have been described, including extended-spectrum serine-beta-lactamas (ESBLs) of groups 2be and 2d (e.g., PER-1 and various OXA-type enzymes) (2,3) and metallo-beta-lactamas of group 3 (e.g., IMP-1 and the recently described VIM-1 and VIM-2 enzymes) (2,4,5). The secondary ESBLs can degrade penicillins, expanded-spectrum cephalosporins, and monobactams (but not carbapenems) and are often susceptible to serine-beta-lactamase inhibitors (1-3). The secondary metallo-beta-lactamas, on the other hand, are notable for their carbapenemase activity and can degrade virtually all beta-lactams except monobactams, while being resistant to the currently available inhibitors (1,2,5,6).

On March 2000, a multidrug-resistant *P. aeruginosa* (isolate VA-182/00) was isolated in pure culture from a bronchial washing of a 58-year-old patient with multiple myeloma. The patient had been admitted 15 days earlier to the Varese University Hospital with a diagnosis of pneumonia and had been treated with piperacillin (0.5 g twice a day) plus piperacillin (2 g three times a day) for 12 days, and then with imipenem/cilastatin (0.5 g three times a day). No cultures of respiratory tract specimens were done earlier in hospitalization. Multiple myeloma had been diagnosed in 1997, and the patient had been treated with multiple cycles of antiproliferative chemotherapy and had received autologous peripheral blood stem cell transplantation. According to clinical records, *P. aeruginosa* had not been isolated previously during this patient’s protracted illness. In vitro susceptibility testing showed that the *P. aeruginosa* isolate was resistant to mezlocillin, ceftazidime, ceft毗me, aztreonam, imipenem, meropenem, gentamicin, tobramycin, netilmicin (MICs, >128 µg/mL), amikacin (MIC, 64 µg/mL), ciprofloxacin and levofloxacin (MICs, >32 µg/mL). Only piperacillin and piperacillin/tazobactam had MIC values slightly lower than the breakpoints for resistance (64 µg/mL and 48/4 µg/mL, respectively), although, considering the normal MICs of piperacillin for susceptible *P. aeruginosa* (2-8 µg/mL), it was evident that the isolate also had considerable biological resistance to these drugs. A double disk-diffusion test, carried out with standard disks placed 20 mm apart (center-to-center), showed synergy between davanulanae and aztreonam. The treatment was changed to piperacillin/tazobactam (4 g four times a day), and a slow recovery ensued over a 30-day period. The patient died 3 months later following a relapse of the underlying malignancy.

The unusually high carbapenem MICs exhibited by VA-182/00 suggested production of a secondary metallo-beta-lactamase, while the synergy between davanulanae and aztreonam suggested production of a secondary serine ESBL. A crude extract of that isolate, assayed spectrophotometrically (7), exhibited imipenem-hydr oxying activity (94 nmol/min/mg protein, inhibited by EDTA) as well as aztreonam-hydrolyzing activity (11 nmol/min/mg protein, resistant to EDTA). Analytic isoelectric focusing (IEF) of the extract, followed by development with the nitrocefin chromogenic substrate (7), showed three bands of beta-lactamase activity of pl s 5.4, 5.6, and 6.3, suggesting the presence of at least three different secondary enzymes. A colony-blot hybridization with probes for the *bla*<sub>MP</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>PER</sub> resistance genes (all of which have been previously detected in an *P. aeruginosa* clinical isolate from the same hospital [8,9; Luzzaro F., unpub. data]) yielded positive results with both the *bla*<sub>VIM</sub> and the *bla*<sub>PER</sub> probes. Amplification of the resistance genes by polymerase chain reaction (PCR) with primers VIM/DIA-f (5'-CAgATTgCCgATggTgTTTgg) and VIM/DIA-r (5'-AggTgggCAgTTgCCAgCAg) for *bla*<sub>VIM</sub> genes (4,5) and BLAPER-f (5'-gggACA(g/A)TC(g/C)(g/T)ATgAAgATgCaTg) and BLAPER-r (5'-ggg(C/T)(g/C)gCTTAgATgTCgATg) for *bla*<sub>PER</sub> genes (9), yielded amplicons of the expected sizes (522 and 966 bp, respectively). Direct amplicon sequencing identified the two beta-lactamase determinants as *bla*<sub>VIM</sub> (5) and *bla*<sub>PER</sub> (10), respectively, a finding consistent with the pl s 5.4 and 5.6 beta-lactama bands detected in IEF (3,5). Conjugative transfer of the resistance determinants to Escherichia coli proved unsuccessful. In a Southern blot analysis of total undigested DNA from VA-182/00, both the *bla*<sub>VIM</sub> and *bla*<sub>PER</sub> probes apparently hybridized to the chromosomal DNA band; no plasmid bands recognized by either probe were detected. A PCR experiment with primers OXA10-f (5'-gggAA-CAAAgAgTTgCtTgCaTg) and OXA105-r (5'-TTagCCAC-CAATgATgCC(C/CITg)TC), suitable for amplification of *bla*<sub>OXA</sub> genes of the OXA-10 group, did not yield an amplicon of the expected size (719 bp), suggesting that the pl 6.3 beta-lactamase band detected by IEF did not correspond to an enzyme of this group.

This is the first observation of a *P. aeruginosa* clinical isolate simultaneously producing a secondary PER-1 ESBL and a secondary metallo-beta-lactamase. The finding, observed in a hospital where both the resistance genes...
(bla\textsubscript{PER-1} and bla\textsubscript{VIM-2}) had been detected separately among clinical isolates, underscores the possibility of the emergence of new threatening combinations of resistance determinants among nosocomial pathogens. In fact, the recruitment of similar resistance determinants within a single \textit{P. aeruginosa} strain can determine a resistance phenotype to virtually all the available antipseudomonal beta-lactams, an occurrence that can be particularly dramatic when, as in the present case, resistance to beta-lactams is associated with resistance against aminoglycosides and fluoroquinolones. In this case, only piperacillin (which appears to be a relatively poor substrate for both enzymes [3,5]) retained moderate activity in vitro and, administered at high dosage in combination with tazobactam, was apparently effective in vivo. Should a similar resistance phenotype disseminate, it might have strategic implications for the development of new beta-lactamase inhibitors and for selection of beta-lactam compounds to associate with inhibitors.

Acknowledgments
This work was supported in part by grant no. FMRX-CT98-0232 from the European Training and Mobility of Researchers Network on metallo-beta-lactamases.

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