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 ß-lactamases 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-lactamases (ESBLs) of groups 2be and 2d (e.g., PER-1 and various OXA-type enzymes) (2,3) and metallo-beta-lactamases 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-lactamases, 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 ciprofloxacin (0.5 g twice a day) plus piperacillin (2 g three times a day) for 12 days, and then with imipenem/clavulanic (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, cefepime, 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 davanulane 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 davanulane and aztreonam suggested production of a secondary serine ESBL. A crude extract of that isolate, assayed spectrophotometrically (7), exhibited imipenem-hydrolyzing 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 pIs 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 blaIMP, blaVIM, and blaPER resistance genes (all of which have been previously detected in P. aeruginosa clinical isolates from the same hospital [8,9; Luzzaro F, unpub. data]) yielded positive results with both the blaVIM and the blaPER probes. Amplification of the resistance genes by polymerase chain reaction (PCR) with primers VIM/DIA-f (5'-CAgATTgCCgATgTgTTTg) and VIM/DIA-r (5'-AggTgggCCATTTCAgCCAgA) for blaVIM genes (4,5) and BLAPER-f (5'-ggAgACC(g)gATC(g)gCT(g)gATAgATTgTgC) and BLAPER-r (5'-ggCC(T)g(g)gCTTCAgATgTgCTgAT) for blaPER genes (9), yielded amplicons of the expected sizes (522 and 966 bp, respectively). Direct ampiclon sequencing identified the two beta-lactamase determinants as blaIMP-1 (5) and blaPER-1 (10), respectively, a finding consistent with the pIs 5.6 and 5.4 beta-lactamase 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 blaVIM and blaPER 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-CAAgAgTTCTCCTgC) and OXA105-r (5'-TTAgCCAC-CAATAgTgCC(C/T)TgC), suitable for amplification of blaOXA 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<sub>PER-1</sub> and bla<sub>VIM-2</sub>) 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 P. aerugi-

nosa 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 combi-
nation 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 comp-
ounds to associate with inhibitors.

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References

10. Nordmann P, Naas T. Sequence analysis of PER-1 extended-

Jamestown Canyon Virus: Seroprevalence in Connecticut

To the Editor: Jamestown Canyon virus (JCV), a member of the California serogroup, has a wide geographic distribution throughout much of temperate North America. It causes mild febrile illness and, rarely, aseptic meningitis or primary encephalitis (1). JCV has been isolated from mosquitoes each year that surveys have been done in Connecticut, and 28 positive pools from 10 mosquito species were found during 2000 (T. Andreadis, pers. commun.). In contrast, only 14 positive mosquito pools were found to contain West Nile virus (WNV), which has recently been introduced into Connecticut (2). JCV has been isolated from Aedes mosquitoes in Con-
necticut, and serologic evidence suggests it is widespread in deer (3,4). No recent seroprevalence surveys have been done in Connecticut, nor have any human cases of infection or dis-
ease due to JCV been documented.

We report the results of two seroprevalence surveys done with standard indirect fluorescent assays (IFA) to detect immunoglobulin G antibodies to JCV. One survey examined 1,086 sera collected in 1990 from blood donors. The second survey examined 1,016 sera submitted to the Connecticut State Public Health Laboratory in 1995.

The IFA used JCV-infected baby hamster kidney cells (BHK-21). Infected and uninfected cell suspensions were air dried and fixed onto Teflon-coated, 12-well slides. Prepared slides were stored at -70°C. Sera were tested at a minimum dilution of 1:16. After incubation and washing of the fluores-
cine-conjugated counterstain, slides were dried and exam-
ined by fluorescent microscope (American Optical, Buffalo, NY). The positive human control serum was designated as the 4+ baseline with which the test sera were compared. Selected sera were tested by a serum dilution plaque reduc-
tion neutralization test (PRNT) assay with JCV, La Crosse virus, and trivittatus virus.

Of the 1,086 sera collected from blood donors in 1990, 164 (15%) were positive by IFA at a minimum dilution of 1:16. Because IFA screening procedures are known to have poor specificity, a subset of 39 IFA-positive and 5 IFA-nega-
tive sera was tested by PRNT. None of the IFA-negative sera were positive, while 26 (67%) of the 39 IFA-positive sera were positive for JCV antibodies. Extrapolating the PRNT results to the 164 IFA-positive sera yields an overall positivity rate of 10.1%.

The second serosurvey, performed on 1,016 sera col-
lected in 1995 from apparently healthy patients requesting immune status testing to viruses such as Varicella zoster or measles, had 57 IFA-positive specimens. Extrapolating addi-