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

To the Editor: In *Pseudomonas aeruginosa*, secondary beta-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/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, 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 davulanate 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 davulanate 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 pl 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 

Letters


Emerging Infectious Diseases

Vol. 7, No. 5, September-October 2001

910
Letters

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 Connecticut, 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 disease 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 fluorescein-conjugated counterstain, slides were dried and examined 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 reduction 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-negative 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 collected 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-

References

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Jean-Denis Docquier,* Francesco Luzzaro,† Gianfranco Amicosante,‡ Antonio Tonio,‡ and Gian Maria Rossolini*‡

*Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, Siena, Italy; †Laboratorio di Microbiologia, Ospedale di Crollo e Università dell’Insubria, Varese, Italy; and ‡Dipartimento di Scienze e Tecnologie Biomediche, Università di L’Aquila, L’Aquila, Italy

Vol. 7, No. 5, September-October 2001 911

Emerging Infectious Diseases