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Carbapenem-Hydrolyzing Metallo-ß-Lactamase from a Nosocomial Isolate of *Pseudomonas aeruginosa* in France

To the Editor: The carbapenems (meropenem and imipenem), the ß-lactams with the broadest spectrum, are stable to most ß-lactamases (1). Therefore, they are often used as antibiotics of last resort for treating nosocomial infections due to gram-negative bacteria resistant to other ß-lactams. Resistance to carbapenems and susceptibility to other ß-lactams in *Pseudomonas aeruginosa* is common as a result of reduced drug accumulation or increased expression of pump efflux (1).

Several extended-spectrum ß-lactamases have been reported in *P. aeruginosa*, but only two, IMP-1 and VIM-1, possess an extended hydrolysis profile that includes carbapenems (2-5). The chromosome-borne and plasmid-mediated carbapenem-hydrolyzing ß-lactamase, IMP-1, has been described in several gram-negative rods, including *P. aeruginosa*, *P. cepacia, Alcaligenes xylosoxydans,* and *Enterobacteriaceae* isolates in Japan (4,6). Recently, a chromosome-borne carbapenem-hydrolyzing ß-lactamase, VIM-1, was reported from a clinical isolate of *P. aeruginosa* in Italy (5), and uncharacterized carbapenem-hydrolyzing ß-lactamases have been reported in the United Kingdom and Portugal (7,8). The weakly related IMP-1 and VIM-1 (31.4% amino acid identity) are both zinc-dependent (metallo-enzymes) and confer resistance to all ß-lactams except monobactams (3,5).

In 1996, a 39-year-old French woman was hospitalized in Marseille for chronic myelogenous leukemia, pancytopenia, and allogeneic bone marrow transplantation. After a 15-day stay in the transplantation unit, fever developed and imipenem and amikacin were administered. Despite this treatment, the patient died of septic shock syndrome 5 days later. Three-day-old blood cultures grew a carbapenem-resistant *P. aeruginosa* isolate. This *P. aeruginosa* COL-1 isolate was resistant to most ß-lactams, including piperacillin/tazobactam, imipenem, meropenem, ceftazidime, cefepime (minimum inhibitory concentrations [MICs] of 128, 32, 16, 64, 32 mg/L, respectively), amikacin, tobramycin, gentamicin, netilmicin, and ciprofloxacin; however, the isolate was susceptible to aztreonam (MIC determination, genetic techniques and ß-lactamase assays are described elsewhere [9]). A sonicate of crude extract of *P. aeruginosa* COL-1 culture showed strong imipenem and meropenem hydrolysis activity (0.7 mU/mg and 1.9 mU/mg; reference *P. aeruginosa* strain <0.05 mU/mg) by UV spectrophotometry with 0.1 mM of substrate, after incubation in 50 mM phosphate buffer at 30°C. This activity was lost when the enzyme extract was preincubated with 10 mM of edetic acid and was partially restored

by addition of 1 mM $ZnCl_2$, indicating the presence of a metallo-carbapenem hydrolyzing ß-lactamase. Isoelectric focusing revealed two ß-lactamase bands of pI 5.6 and 9. Only the pI 5.6 ß-lactamase band was inhibited if the gel was overlaid with edetic acid before nitrocefin was added as the indicator substrate; the other pI 9 ß-lactamase likely corresponded to a naturally occurring AmpC cephalosporinase. This pI 5.6 value differed from the pI values of the carbapenem-hydrolyzing ß-lactamase previously reported in *P. aeruginosa* (3-5,7,8). Polymerase chain reaction amplification experiments were negative when internal primers were used for the only sequenced carbapenem-hydrolyzing ß-lactamase genes from *P. aeruginosa* encoding IMP-1 and VIM-1 and genomic DNA of *P. aeruginosa* COL-1. Transfer of the carbapenem resistance marker by conjugation to laboratory strains of *P. aeruginosa* or *Escherichia coli* was unsuccessful (9), but transformation by electroporation of a putative plasmid extract from *P. aeruginosa* COL-1 in *E. coli*, followed by selection onto amoxicillin-containing agar plates (9), gave a ca. 45-kb plasmid that produced the carbapenem-hydrolyzing ß-lactamase with a pI value of 5.6. Thus, the carbapenem-hydrolyzing ß-lactamase gene was plasmid-borne.

This case indicates the presence of a novel carbapenem-hydrolyzing ß-lactamase in *P. aeruginosa* in Europe, the first in France; its spread in gram-negative rods, as reported for IMP-1 in Japan, is of concern because, as seen in this case, routine laboratory detection is difficult and therapeutic options are extremely limited.

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Population-Based Study of Invasive *Kingella kingae* Infections

To the Editor: For most of the 3 decades since the first description of *Kingella kingae*, this gram-negative bacillus was considered a rare cause of human disease (1). Since the late 1980s, however, reports of infections by the organism in young children have increased in the United States, Western Europe, and Israel (2-6). The rapid emergence of *K. kingae* as an important cause of pediatric disease does not necessarily imply that the organism is truly a new pathogen. Better isolation techniques and awareness of the bacterium by microbiology laboratories may contribute to the apparent increase (4). Recent studies have demonstrated that primary isolation of *K. kingae* can be substantially improved by injection of synovial fluid and bone exudates into aerobic blood-culture bottles (4). Synovial fluid may inhibit the growth of *K. kingae,* and injection of the clinical specimen