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First Report of the Metallo-β-Lactamase SPM-1 in Europe

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In 1997, the first SPM-1-positive *Pseudomonas aeruginosa* isolate (48-1997A) from a 4-year-old leukemic girl in San Paulo, Brazil, was characterized. bla_{SPM-1} was flanked by two IS*CR* elements, designated IS*CR4*, and probably transposes via a mechanism called rolling-circle replication (8). Reports of IS*CR* elements are continually being linked with antibiotic resistance, particularly β-lactamases in Gram-negative bacteria (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr_elements.html) (3, 4, 8).

In 2007, a single isolate of *P. aeruginosa*, designated BH121, was recovered from a soft-wound infection of a 34-year-old Swiss male. The patient received primary care at the Regional General Hospital in Recife, Brazil, before being transported to the University Hospital Basel. *P. aeruginosa* BH121 gave a positive result with the MBL Etest (AB BioMérieux, Solna, Sweden). MICs were determined by agar dilution in accordance with CLSI guidelines (2). BH121 was resistant to all antibiotics (piperacillin-tazobactum, ceftazidime, cefotaxime, cefepime, meropenem, imipenem, all aminoglycosides, and all fluoroquinolones) except aztreonam and colistin. Imipenem hydrolysis by crude cell extracts from BH121 suggested the presence of an active metallo-β-lactamase (MBL) (7).

PCR analysis detected bla_{SPM-1} and was thus extended to determine flanking sequences, which showed that the MBL gene is flanked by two copies of IS*CR4*-like elements. Sequence analysis confirmed that BH121 carries bla_{SPM-1} and that it possesses two copies of IS*CR4* identical to that reported by Poirel et al., indicating perfect preservation of this DNA region for >10 years (5, 7, 10).

DNA macroanalysis using pulsed-field gel electrophoresis (PFGE) after restriction with SpeI was undertaken to ascertain the relatedness of BH121 to 15 other SPM-1-positive *P. aeruginosa* isolates from Brazil between 1997 and 2007 (6). This analysis showed that BH121 had a DNA restriction pattern almost identical to that of the other Brazilian strains, differing by only three or four bands, thus indicating that all isolates of *P. aeruginosa* are distantly related.

Genomic DNAs from *P. aeruginosa* BH121 and the 15 retrospective Brazilian isolates were digested with SpeI and probed with bla_{SPM-1}. Probing of SpeI genomic DNA digests of the clinical *P. aeruginosa* isolates with bla_{SPM-1} shows two copies of the MBL gene in three strains, including *P. aeruginosa* BH121 and the index case strain, 48-1997. The duplication of bla_{SPM-1} is likely to have arisen from IS*CR4* transposition proceeded by homologous recombination.

To determine the genetic location of the MBL gene, genomic DNA from all isolates was digested separately with nucleases S1 and I-Ceu-1, separated by PFGE, and subsequently probed with bla_{SPM-1} (1). Data showed that the bla_{SPM-1} probe hybridized only to chromosomal DNA and not the separated plasmid bands, indicating that the MBL gene is chromosomally mediated. DNA digestion with I-Ceu-1 and probing with bla_{SPM-1} also confirmed that the gene is chromosomally encoded.

This is the first reported case of a *P. aeruginosa* isolate possessing bla_{SPM-1} outside Brazil and shows that BH121 is closely related to isolates originating from Brazil. Moreover, bla_{SPM-1} probing of digested DNA indicates that the SPM-1 gene is chromosomally mediated and, interestingly, that there are at least two copies of bla_{SPM-1} in *P. aeruginosa* BH121, indicating that IS*CR4* is likely to be active (9).

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