

## First Report of the Metallo- $\beta$ -Lactamase SPM-1 in Europe<sup>∇</sup>

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*<sub>SPM-1</sub> was flanked by two ISCR elements, designated ISCR4, and probably transposes via a mechanism called rolling-circle replication (8). Reports of ISCR elements are continually being linked with antibiotic resistance, particularly  $\beta$ -lactamases in Gram-negative bacteria ([http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr\\_elements.html](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-tazobactam, 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- $\beta$ -lactamase (MBL) (7).

PCR analysis detected *bla*<sub>SPM-1</sub> and was thus extended to determine flanking sequences, which showed that the MBL gene is flanked by two copies of ISCR4-like elements. Sequence analysis confirmed that BH121 carries *bla*<sub>SPM-1</sub> and that it possesses two copies of ISCR4 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*<sub>SPM-1</sub>. Probing of SpeI genomic DNA digests of the clinical *P. aeruginosa* isolates with *bla*<sub>SPM-1</sub> 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*<sub>SPM-1</sub> is likely to have arisen from ISCR4 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*<sub>SPM-1</sub> (1). Data showed that the *bla*<sub>SPM-1</sub> 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*<sub>SPM-1</sub> also confirmed that the gene is chromosomally encoded.

This is the first reported case of a *P. aeruginosa* isolate possessing *bla*<sub>SPM-1</sub> outside Brazil and shows that BH121 is closely related to isolates originating from Brazil. Moreover, *bla*<sub>SPM-1</sub> probing of digested DNA indicates that the SPM-1 gene is chromosomally mediated and, interestingly, that there

are at least two copies of *bla*<sub>SPM-1</sub> in *P. aeruginosa* BH121, indicating that ISCR4 is likely to be active (9).

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<sup>∇</sup> Published ahead of print on 26 October 2009.