Clinical Carbenapenem-Resistant Acinetobacter baylyi Strain Coharboring \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) from China

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Carbenapenem-resistant Acinetobacter spp. are one of the most frightening threats in the current antibiotic era (14). The mechanisms of Acinetobacter sp. resistance to carbenapenem are production of carbenapenemases (the main mechanism), overexpression of efflux pumps, and reduced expression or loss of outer membrane proteins (8). The metallo-\( \beta \)-lactamase (MBL) gene, \( \text{bla}_{\text{SIM-1}} \), was first identified in Acinetobacter spp. from South Korea in 2005 (11) and then in Acinetobacter genomic species 10 from two hospitals of South Korea in 2010 (10) but, as far as we know, has not yet been detected in other countries. Here, we report the emergence of Acinetobacter baylyi coharboring \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) in China.

A 59-year-old male was admitted for cerebral hemorrhage at the Affiliated Hospital of Ningbo University in June 2009. After surgery to remove the intracerebral hematoma, he was transferred to the intensive care unit and received antibiotic therapy with cefminox, which was later replaced with cefodizime plus amikacin. Imipenem-resistant Acinetobacter sp. NB09A30 was isolated from the cloudy secretion of the surgical incision 10 days after the operation. MICs were determined by Etest and interpreted according to the CLSI 2011 recommendation (7). The strain was resistant to almost all \( \beta \)-lactams, including imipenem (MIC ≥ 32 \( \mu \)g/ml) and meropenem (MIC ≥ 32 \( \mu \)g/ml), but remained susceptible to minocycline, tigecycline, ciprofloxacin, levofloxacin, and colistin. The MICs of gentamicin and amikacin were ≥256 \( \mu \)g/ml and 16 \( \mu \)g/ml, respectively. Etest MBL strip testing against NB09A30 indicated the presence of metallo-\( \beta \)-lactamase (imipenem/imipenem plus EDTA MIC of >16 \( \mu \)g/ml). Surgical site infection was suspected, and the antibiotic regimen was changed to levofloxacin. The man was cured finally, and NB09A30 was no longer recovered.

PCR detection of the \( \text{bla}_{\text{OXA-51}} \)-like gene in NB09A30 was negative, implying that NB09A30 is not an Acinetobacter baumannii strain (15). The 16S-23S rRNA intergenic spacer region (ISR) of NB09A30 was amplified and cloned into the pGEM-T Easy vector for sequencing (5). This showed that NB09A30 possessed ISRs with different lengths (long ISR, 650 bp; short ISR, 611 bp), a unique organization of the 16S-23S rRNA ISRs of A. baylyi (12). The ISR sequences were 94% identical to those of A. baylyi strain 93A2 (GenBank accession no. EU042163) and A. baylyi ADP1 (GenBank accession no. CR543861); they were less than 85% identical to those of the other genomic species, which indicated that NB09A30 is an A. baylyi strain (12).

PCR was performed to check for the presence of carbenapenem-hydrolyzing class D \( \beta \)-lactamase (CHDL) genes (\( \text{bla}_{\text{OXA-23}} \), \( \text{bla}_{\text{OXA-24}} \), and \( \text{bla}_{\text{OXA-58}} \)). MBL genes (\( \text{bla}_{\text{IMP}} \)-type and \( \text{bla}_{\text{VIM}} \)-type genes, \( \text{bla}_{\text{SIM-1}} \), \( \text{bla}_{\text{SIM-1}} \), \( \text{bla}_{\text{GIM-1}} \), and \( \text{bla}_{\text{SIM-1}} \)), and the \( \text{bla}_{\text{KPC}} \)-type gene, as previously reported (13, 16), \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) were detected and confirmed by sequencing.

Repeated mating-out assays and electrotransformation to transfer \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) failed. Therefore, we used an S1 nuclease assay and an I-CeuI assay to identify the locations of the two carbenapenemase genes as previously described (2). Two 23S rRNA gene probes were designed to test the sequences upstream and downstream of the I-CeuI cut site, respectively, according to the genomic sequence of A. baylyi strain ADP1 (GenBank accession no. CR543861). All seven I-CeuI-generated DNA fragments can be hybridized with 23S rRNA gene probe 1 plus 23S rRNA gene probe 2 but did not cohybridize with \( \text{bla}_{\text{SIM-1}} \) or \( \text{bla}_{\text{OXA-23}} \) probes, excluding chromosomal locations for the two carbenapenemase genes (Fig. 1).

In contrast, hybridization signals of \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) probes were detected in a ca. 360-kb large plasmid after S1 nuclease treatment, suggesting plasmid locations for the two carbenapenemase genes (Fig. 1). \( \text{bla}_{\text{SIM-1}} \) was confirmed to have a chromosomal location in Acinetobacter spp. of South Korea (10, 11), but it has a large-plasmid location in A. baumannii strain NB09A30. Using the A. baumannii plasmid replicon typing scheme constructed by Bertini et al. (4), we detected four replicate (rep) gene groups (GRs), GR2, GR3, GR7, and...
lates from the same hospital. Multilocus sequence typing (MLST) identified an identical sequence type, ST138 (http://pubmlst.org/abaumannii/), in all isolates (3); this sequence type was clustered into clonal complex 92 (CC92), the dominant clonal complex of carbapenem-resistant A. baumannii in China (9). blaOXA-23 was identified in all isolates, but blaSIM-1 was absent. The patient in this case had no history of visiting South Korea. Moreover, we failed to detect blaSIM-1 in A. baumannii isolates prevalent in the hospital at the time. Therefore, the source of blaSIM-1 in A. baylyi NB09A30 is still unknown. The genetic structure of blaOXA-23 in the prevalent A. baumannii isolates with ST138 included Tn2009, a novel blaOXA-23-containing transposon also identified in CC92 A. baumannii strain MDR-ZJ06 from China (17). Distinct from Tn2009, which was flanked on one side by IS4aba1, Tn2009 was a composite transposon flanked by two IS4aba1 sequences with identical orientations (17), implying different origins of blaOXA-23 in A. baylyi NB09A30 and A. baumannii ST138.

In conclusion, our study identified blaSIM-1 for the first time in China. Since the potential source and details of the epidemiological situation are unclear, more study should be carried out to survey the prevalence of blaSIM-1 in Acinetobacter spp. A. baylyi used to be considered an environment species, existing in soil and activated sludge, and nonpathogenic (8). Though nosocomial infections caused by A. baylyi have been reported (6), carbapenem resistance is still rare. More attention should be paid to the emergence of carbapenem resistance in this potential pathogen.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S–23S rRNA intergenic spacer region, the blaSIM-1-containing class I integron, Tn2008 of A. baumannii strain NB09A30, and Tn2009 of the prevalent A. baumannii isolates of ST138 are deposited in the GenBank database under accession no. JF731031 to JF731040, JF731030, JF731029, and JF731028, respectively.

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