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# Clinical Carbapenem-Resistant *Acinetobacter baylyi* Strain Coharboring $bla_{SIM-1}$ and $bla_{OXA-23}$ from China▽

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**$bla_{SIM-1}$ and $bla_{OXA-23}$ were codetected in clinical carbapenem-resistant *Acinetobacter baylyi* strain NB09A30. Both of carbapenemase genes were located on a large plasmid (ca. 360 kb). $bla_{SIM-1}$ was found as a gene cassette inserted into a class 1 integron identical to that determined in *Acinetobacter* sp. isolates from South Korea. The genetic structure of $bla_{OXA-23}$ in NB09A30 was different from that in the prevalent *Acinetobacter baumannii* of clonal complex 92 (CC92) from the same hospital.**

Carbapenem-resistant *Acinetobacter* spp. are one of the most frightening threats in the current antibiotic era (14). The mechanisms of *Acinetobacter* sp. resistance to carbapenem are production of carbapenemases (the main mechanism), overexpression of efflux pumps, and reduced expression or loss of outer membrane proteins (8). The metallo-β-lactamase (MBL) gene, $bla_{SIM-1}$, 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 $bla_{SIM-1}$ and $bla_{OXA-23}$ 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 β-lactams, including imipenem (MIC ≥ 32 μg/ml) and meropenem (MIC ≥ 32 μg/ml), but remained susceptible to minocycline, tigecycline, ciprofloxacin, levofloxacin, and colistin. The MICs of gentamicin and amikacin were ≥256 μg/ml and 16 μg/ml, respectively. Etest MBL strip testing against NB09A30 indicated the presence of metallo-β-lactamase (imipenem/imipenem plus EDTA MIC of >16 μg/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 $bla_{OXA-51}$-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 carbapenem-hydrolyzing class D β-lactamase (CHDL) genes ($bla_{OXA-23}$, $bla_{OXA-24}$, and $bla_{OXA-58}$), MBL genes ($bla_{IMP}$-type and $bla_{VIM}$-type genes, $bla_{SIM-1}$, $bla_{SPM-1}$, $bla_{GIM-1}$, and $bla_{NDM-1}$), and the $bla_{KPC}$-type gene, as previous reported (13, 16). $bla_{SIM-1}$ and $bla_{OXA-23}$ were detected and confirmed by sequencing.

Repeated mating-out assays and electrotransformation to transfer $bla_{SIM-1}$ and $bla_{OXA-23}$ failed. Therefore, we used an S1 nuclease assay and an I-CeuI assay to identify the locations of the two carbapenemase genes as previously described (2). Two 23S rRNA gene probes were designed to target 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 $bla_{SIM-1}$ or $bla_{OXA-23}$ probes, excluding chromosomal locations for the two carbapenemase genes (Fig. 1). In contrast, hybridization signals of $bla_{SIM-1}$ and $bla_{OXA-23}$ probes were codetected in a ca. 360-kb large plasmid after S1 nuclease treatment, suggesting plasmid locations for the two carbapenemase genes (Fig. 1). $bla_{SIM-1}$ was confirmed to have a chromosomal location in *Acinetobacter* spp. of South Korea (10, 11), but it has a large-plasmid location in *A. baylyi* strain NB09A30. Using the *A. baumannii* plasmid replicon typing scheme constructed by Bertini et al. (4), we detected four replicase (rep) gene groups (GRs), GR2, GR3, GR7, and

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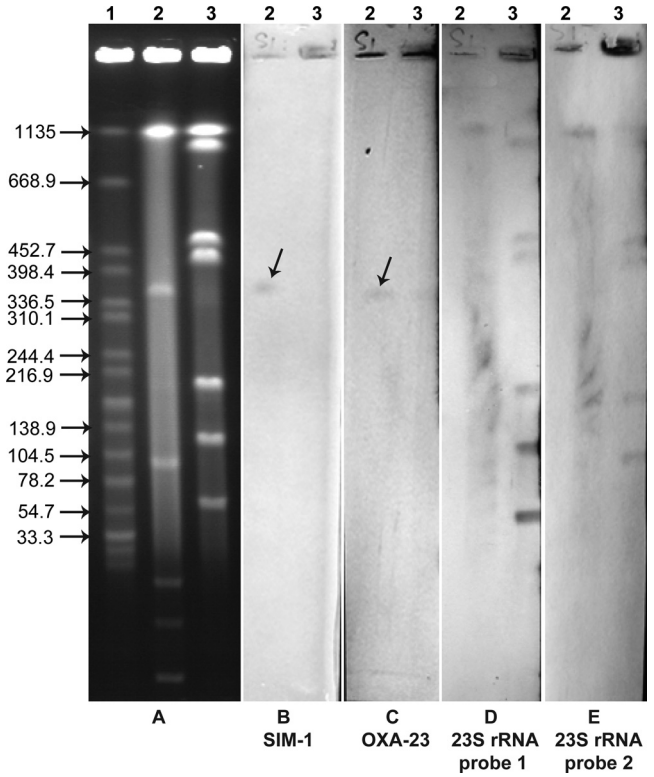


FIG. 1. Analysis of the locations of $bla_{SIM-1}$ and *bla*OXA-23 in *A. baylyi* strain NB09A30 using the S1 nuclease assay and I-CeuI assay. Shown are pulsed-field gel electrophoresis (PFGE) profiles of total DNA after digestion (A) and Southern blotting and hybridization results with $bla_{SIM-1}$ (B), $bla_{OXA-23}$ (C), 23S rRNA gene probe 1 (D), and 23S rRNA gene probe 2 (E). The largest chromosomal fragment of the I-CeuI profile was not hybridized using 23S rRNA gene probe 1 (D). The smallest chromosomal fragment was not hybridized using 23S rRNA gene probe 2 (E). Arrows indicate the hybridization signals of $bla_{SIM-1}$ and $bla_{OXA-23}$. Lane 1, *Salmonella enterica* serotype Braenderup strain H9812 DNA digested by XbaI, used as a molecular marker (kb); lanes 2, *A. baylyi* strain NB09A30 digested by S1 nuclease; lanes 3, *A. baylyi* strain NB09A30 DNA digested by I-CeuI.

GR13, in *A. baylyi* strain NB09A30. Further hybridization with corresponding probes is needed to define the large plasmid's rep group.

Primers targeting 5′ and 3′ conserved segments of the class I integron were used for PCR amplification (11). $bla_{SIM-1}$ of *A. baylyi* strain NB09A30 was found to be class I integron borne. It is interesting that the sequence of the entire integron was 100% identical to that in *Acinetobacter* sp. isolates from South Korea (GenBank accession no. EF125010) (11). The genetic structure of $bla_{OXA-23}$ was determined by a cloning experiment as previously reported (1). $bla_{OXA-23}$ in *A. baylyi* strain NB09A30 was part of transposon Tn*2008* (1), which was inserted into a noncoding region and flanked by two copies of an 11-bp direct repeat (ATATTCTGTTT).

In order to trace the source of $bla_{SIM-1}$ and $bla_{OXA-23}$, we further screened for the presence of the two carbapenemases in clinical *A. baumannii* isolates. From October 2008 to June 2009, we collected 29 carbapenem-resistant *A. baumannii* isolates 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). $bla_{OXA-23}$ was identified in all isolates, but $bla_{SIM-1}$ was absent. The patient in this case had no history of visiting South Korea. Moreover, we failed to detect $bla_{SIM-1}$ in *A. baumannii* isolates prevalent in the hospital at the time. Therefore, the source of $bla_{SIM-1}$ in *A. baylyi* NB09A30 is still unknown. The genetic structure of $bla_{OXA-23}$ in the prevalent *A. baumannii* isolates with ST138 included Tn*2009*, a novel $bla_{OXA-23}$-containing transposon also identified in CC92 *A. baumannii* strain MDR-ZJ06 from China (17). Distinct from Tn*2008*, which was flanked on one side by IS*Aba1*, Tn*2009* was a composite transposon flanked by two IS*Aba1* sequences with identical orientations (17), implying different origins of $bla_{OXA-23}$ in *A. baylyi* NB09A30 and *A. baumannii* ST138.

In conclusion, our study identified $bla_{SIM-1}$ 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 presence of $bla_{SIM-1}$ 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 $bla_{SIM-1}$-containing class I integron, Tn*2008* of *A. baylyi* strain NB09A30, and Tn*2009* 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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