A Clinical Carbapenem-Resistant Acinetobacter baylyi Strain
Co-harboring $\text{bla}_{\text{SIM-1}}$ and $\text{bla}_{\text{OXA-23}}$ from China

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Running title: $\text{bla}_{\text{SIM-1}}$ and $\text{bla}_{\text{OXA-23}}$ harboring Acinetobacter baylyi

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Abstract

$\text{bla}_{\text{SIM-1}}$ and $\text{bla}_{\text{OXA-23}}$ were co-detected in a clinical carbapenem-resistant \textit{Acinetobacter baylyi} strain NB09A30. Both of carbapenemase genes were located on a ca. 360 kb large plasmid. $\text{bla}_{\text{SIM-1}}$ was found as a gene cassette inserted into a class 1 integron identical to that determined in \textit{Acinetobacter} spp. isolates from South Korea. The genetic structure of $\text{bla}_{\text{OXA-23}}$ in NB09A30 was different from that in the prevalent \textit{Acinetobacter baumannii} of CC92 from the same hospital.
Carbapenem-resistant *Acinetobacter* spp. is one of the most frightening threats to the current antibiotic era (14). The resistant mechanisms of *Acinetobacter* spp. against carbapenem involve production of carbapenemases, overexpression of efflux pumps as well as reduced expression or loss of out membrane proteins, in all of which carbapenemases producing are considered as the main one (7). The metallo-β-lactamase, *bla*SIM-1, was firstly 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 reported the emergence of *bla*SIM-1 and *bla*OXA-23 co-harboring *Acinetobacter baylyi* 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 intensive care unit and received antibiotic therapy with cefminox, and then replaced with cefodizime plus amikacin. Imipenem-resistant *Acinetobacter* spp. NB09A30 was isolated from the cloudy secretion of surgical incision after 10 days of operation. Minimal inhibitory concentrations were determined by Etest and interpreted according to the CLSI 2011 recommendation (9). It was resistant to almost all β-lactams including imipenem (≥32 μg/ml) and meropenem (≥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+EDTA >16). Surgical site infection was suspected and the antibiotic regimen was changed to levofloxacin. The man was cured finally and the same strain was no longer recovered.
PCR detection of bla\textsubscript{OXA-51}-like in NB09A30 was negative implying it a non-Acinetobacter baumannii species (15). The 16S-23S rRNA intergenic spacer region (ISR) of NB09A30 was amplified and cloned into pGEM-T Easy vector for sequencing (5). It showed NB09A30 possessed ISRs with different length (long ISR, 650 bp; short ISR, 611 bp), a unique organization of the 16S-23S rRNA ISR of \textit{A. baylyi} (12). The ISRs sequences were 94% identity with that of \textit{A. baylyi} strain 93A2 (GenBank accession No. EU042163) and \textit{A. baylyi} ADP1 (GenBank accession No. CR543861), while were less than 85% identity with the other genomic species, which indicated NB09A30 as an \textit{A. baylyi} strain (12).

PCR was performed for the presence of CHDLs (bla\textsubscript{OXA-23}-like, bla\textsubscript{OXA-24}-like and bla\textsubscript{OXA-58}), MBLs (bla\textsubscript{IMP}-type, bla\textsubscript{VIM}-type, bla\textsubscript{SIM-1}, bla\textsubscript{SPM-1}, bla\textsubscript{GIM-1} and bla\textsubscript{NDM-1}) and bla\textsubscript{KPC}-type as previous report (13, 16). bla\textsubscript{SIM-1} and bla\textsubscript{OXA-23} were detected and confirmed by sequencing.

Repeated mating-out assay and electrotransformation to transfer bla\textsubscript{SIM-1} and bla\textsubscript{OXA-23} were failed. Therefore, we used an S1 nuclease assay and an I-CeuI assay to identify the location of the two carbapenemase genes as previously described (2). Two 23S rRNA gene probes were designed targeted the upstream and downstream sequences of I-CeuI cut site respectively according to the genomic sequence of \textit{A. baylyi} strain ADP1 (GenBank accession No. CR543861). All seven I-CeuI-generating DNA fragments can be hybridized with 23S rRNA gene probe 1 plus 23S rRNA gene probe 2, but not co-hybridized with bla\textsubscript{SIM-1} or bla\textsubscript{OXA-23} probes, excluding the chromosomal location of the two carbapenemase genes (Fig. 1). In contrast, hybridization signals of bla\textsubscript{SIM-1} and bla\textsubscript{OXA-23} probes were co-detected in a ca. 360 kb large plasmid after S1 nuclease treatment, suggesting plasmid mediated of the two carbapenemase genes (Fig. 1). bla\textsubscript{SIM-1} was confirmed chromosomal location in
Acinetobacter spp. of South Korea (10, 11), while 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 rep groups, gr2, gr3, gr7 and 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 class I integron were used for PCR amplification (11). blaSIM-1 of A. baylyi strain NB09A30 was found class I integron-borne. It is interesting that the sequence of the entire integron was 100% identity with that in Acinetobacter spp. isolates from South Korea (GenBank accession No. EF125010) (11). The genetic structure of blaOXA-23 was determined by cloning experiment as previously reported (1). blaOXA-23 in A. baylyi strain NB09A30 was part of transposon Tn2008 (1), which was inserted into a non-coding region and flanked by two copies of an 11-bp direct repeat (ATATTCTGTTT).

In order to trace the source of blaSIM-1 and blaOXA-23, we further screened the presence of the two carbapenemases in the clinical A. baumannii. From Oct 2008 to Jun 2009, we collected 29 carbapenem-resistant A. baumannii isolates from the same hospital. MLST identified an identical sequence type ST138 (http://pubmlst.org/abaumannii/) in all isolates (3), which was clustered into CC92, the dominant clonal complex of carbapenem-resistant A. baumannii in China (8). blaOXA-23 was identified in all isolates, but blaSIM-1 was absent. The patient in this case had no South Korea visit history. Moreover, we failed to detected blaSIM-1 in the contemporary hospital-prevalent A. baumannii. Therefore, the source of blaSIM-1 in A. baylyi NB09A30 is still unknown. The genetic structure of blaOXA-23 in the prevalent A. baumannii of ST138 was Tn2009, a novel blaOXA-23-containing transposon also identified in another CC92 A. baumannii strain MDR-ZJ06 from China (17). Distinct
from one-side flanked ISAba1 in Tn2008, Tn2009 was a composite transposon flanked by two ISAba1 with identical orientation (17), implying different origin of blaOXA-23 in A. baylyi NB09A30 and A. baumannii ST138.

In conclusion, our study firstly identified blaSIM-1 in China. Since the potential source and the detail epidemiological situation are unclear, more study should be carried out to survey the presence of blaSIM-1 in Acinetobacter spp., A. baylyi used to be considered as an environment species existing in soil and activated sludge and non-pathogenic (7). Though nosocomial infections caused by A. baylyi has 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, blaSIM-1-containing class I integron, Tn2008 of A. baylyi strain NB09A30 and Tn2009 of the prevalent A. baumannii of ST138 are deposited in the GenBank database under accession No. JF731031 to JF731040, JF731030, JF731029 and JF731028 respectively.

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Fig. 1. Analysis of the location of \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \) in \textit{A. baylyi} strain NB09A30 using S1-nuclease assay and I-CeuI assay. PFGE profiles of total DNA after digestion (A); Southern blotting and hybridization results with \( \text{bla}_{\text{SIM-1}} \) (B), \( \text{bla}_{\text{OXA-23}} \) (C), 23S rRNA gene probe 1 (D) and 23S rRNA gene probe 2 (E). Using 23S rRNA gene probe 1, the largest chromosomal fragment of I-CeuI profile was not hybridized (D). Using 23S rRNA gene probe 2, the smallest chromosomal fragment was not hybridized (E). The black arrow indicates the hybridization signal of \( \text{bla}_{\text{SIM-1}} \) and \( \text{bla}_{\text{OXA-23}} \). Lane 1: \textit{Salmonella} serotype Braenderup strain H9812 digested by XbaI used for molecular marker (kb); Lane 2: \textit{A. baylyi} strain NB09A30 digested by S1 nuclease; Lane 3: \textit{A. baylyi} strain NB09A30 digested by I-CeuI.
Fig. 1