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Imipenem-Susceptible, Meropenem-Resistant *Klebsiella pneumoniae* Producing OXA-181 in Japan

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The incidence of infections by *Enterobacteriaceae* carrying the carbapenemase gene showing a paradoxical phenotype of resistance to virtually all ß-lactams, including meropenem but not including imipenem, is increasing (1, 2, 3). Here we report the first isolation of *Klebsiella pneumoniae* carrying $bla_{\text{OXA-181}}$ in Japan showing a similar stealth-type resistance phenotype.

In April 2010, a man was admitted to a hospital in Mumbai, India, with unidentified multiple organ failures. In June 2010, he was transferred to an intensive care unit (ICU) in Hiroshima, Japan. *K. pneumoniae* MS5166 was isolated from the urine sample of the patient. This isolate was resistant to almost all ß-lactams, including meropenem, but was susceptible to imipenem following CLSI criteria (4): the MIC was 2 μg/ml for imipenem and 8 μg/ml for meropenem using the broth microdilution method. Further, MS5166 was resistant to aminoglycosides and fluoroquinolones. The MIC values (μg/ml) were as follows: for amikacin, >32; for gentamicin, >8; for tobramycin, >8; for ciprofloxacin, >2; and for levofloxacin, >4. The result of the metallo-β-lactamase phenotype test (5) was negative. PCR for carbapenemase genes performed using 11 universal primer sets (6) gave a positive result for the gene encoding OXA-48-like protein. Direct sequencing of the amplicon indicated it is 100% identical to $bla_{\text{OXA-181}}$, differing from $bla_{\text{OXA-48}}$ by four amino acid substitutions.

The draft genome sequence of strain MS5166 generated using Illumina MiSeq (Nextera paired-end library; 3,971,486 bp; 62.33-fold coverage), assembled using CLC Genomics Workbench (CLC bio, Cambridge, MA), and generated using OSLay (7) indicated that $bla_{\text{OXA-181}}$ is on the chromosome (Fig. 1).

IS*Ecp1* is located upstream of $bla_{\text{OXA-181}}$ as reported previously (8, 9). $bla_{\text{OXA-181}}$ is located on a deleted version of Tn*2013* (2,961 bp), flanked by a 5-bp duplication of the target site (AAAGA) in the MS5166 chromosome between the arginine ABC transporter substrate-binding protein gene and the *yjeS* gene (Fig. 1). A putative inverted repeat right (IRR) of IS*Ecp1* revealed weak identity with its original IRR sequence (8). Potron et al. previously showed

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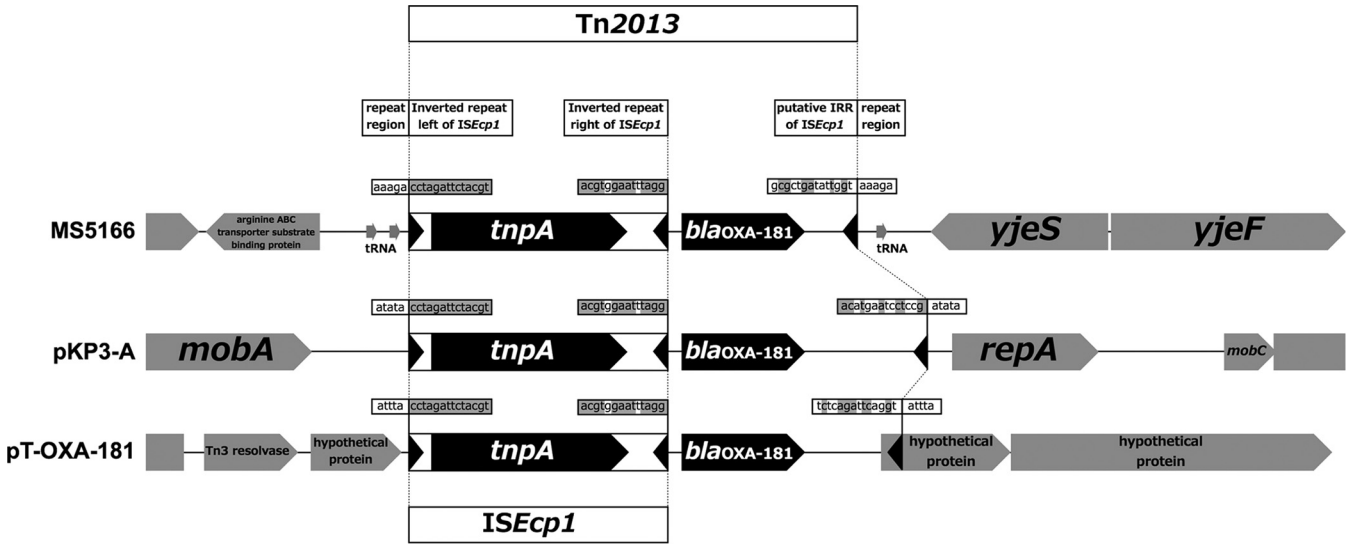

FIG 1 Structural comparison of strains MS5166, pKP3-A, and pT-OXA-181 in the IS*Ecp1*-$bla_{\text{OXA-181}}$ region (GenBank accession no. AB972272, JN205800, and JQ996150, respectively). Open reading frames (ORFs) are represented by pentagons. Nucleotide letters in squares represent direct or inverted repeats (shaded base pairs are identical, and white base pairs are different), and target site duplications (repeat regions) are represented in squares. Tn*2013* in MS5166 was found to be located between bp 438346 and bp 438347 on the chromosome of the *K. pneumoniae* NTUH-K2044 genome (GenBank accession no. NC012731).

that Tn*2013* integrated into the *Escherichia coli* chromosome upon conjugation *in vitro* (8). This is the first clinical case of chromosomally integrated Tn*2013* shown to be IS*Ecp1* associated with the $bla_{OXA-181}$ gene in *K. pneumoniae*.

A ResFinder search (10) of the draft genome sequence of MS5166 identified $bla_{CTX-M-15}$, bla_{TEM-1A}, bla_{SHV-11}, *armA*, *aadA2*, *aac(6′)-Ib*, *aadA1*, and *aac(6′)Ib-cr* genes in addition to the $bla_{OXA-181}$ gene. Multilocus sequence typing showed that MS5166 belongs to ST43, a "minor" cluster producing OXA-181 and CTX-M-15 in India (11).

The catalytic activities (k_{cat}/K_m) of OXA-181 for imipenem were reportedly 20 times higher than those for meropenem (12). We therefore sought a mechanism other than OXA-181 to explain the imipenem-susceptible, meropenem-resistant phenotype of MS5166. We identified a 14-bp deletion in *ompK35* leaving a 22-amino-acid sequence with a truncation of the large C-terminal region and a frameshift mutation in *ompK36* causing addition of two amino acids in loop 3 generating the variant OmpK36V (13). A clinical *K. pneumoniae* isolate producing extended-spectrum β-lactamase (ESBL) and OmpK36V but lacking OmpK35 showed the imipenem-susceptible, meropenem-resistant phenotype (13). Therefore, the absence of OmpK35 and the frameshift mutation in OmpK36 may be the mechanisms for this peculiar carbapenem resistance phenotype in ESBL- and OXA-181-producing *K. pneumoniae* MS5166.

Nucleotide sequence accession number. The partial sequence of MS5166 containing $bla_{OXA-181}$ was deposited in GenBank (accession no. AB972272).

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