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First Report of KPC-2 Carbapenemase-Producing *Klebsiella pneumoniae* in Japan

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We investigated a novel Japanese isolate of sequence type 11 (ST11), the *Klebsiella pneumoniae* carbapenemase-2 (KPC-2)-producing *K. pneumoniae* strain Kp3018, which was previously obtained from a patient treated at a Brazilian hospital. This strain was resistant to various antibiotic classes, including carbapenems, and harbored the gene bla_{KPC-2}, which was present on the transferable plasmid of ca. 190 kb, in addition to the $bla_{CTX-M-15}$ gene. Furthermore, the ca. 2.3-kb sequences (IS*Kpn8*-bla_{KPC-2}–IS*Kpn6*-like), encompassing bla_{KPC-2}, were found to be similar to those of *K. pneumoniae* strains from China.

The increase in carbapenemase-producing *Enterobacteriaceae* is of serious concern globally, including in Japan, because these organisms are resistant to the carbapenems that are used to treat severe infections caused by multidrug-resistant bacteria (1–3). Ambler class A (*Klebsiella pneumoniae* carbapenemase [KPC]), class B (IMP, VIM, and NDM-1), and class D (OXA-48) β-lactamases are known as carbapenemases (1, 3). In particular, since KPC-producing *K. pneumoniae* isolates were first reported in the United States in 2001 (4), KPC-producing *Enterobacteriaceae* have been isolated as a cause of nosocomial and community-acquired infections in various countries (1, 3). However, the isolation of KPC-producing bacteria has not yet been described in Japan. In the present study, we investigated a novel Japanese isolate of KPC-2-producing *K. pneumoniae* strain Kp3018, isolated from a patient previously treated at a Brazilian hospital.

A 73-year-old Japanese man was admitted to a Brazilian hospital for a sudden onset of cerebral hemorrhage on 23 May 2012. On 4 July 2012, he was transferred to Tokyo and admitted to the Medical Hospital of Tokyo Medical and Dental University. Kp3018, a *K. pneumoniae* strain, which is resistant to carbapenems, was isolated from blood, stool, and decubitus ulcer samples from the patient. The MICs of antibiotics were determined using the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Inc., Tokyo, Japan) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (5). The MICs of cefotaxime and ceftazidime with clavulanic acid (4 μg/ml) and of imipenem with dipicolinic acid (400 μg/ml) were also determined by the broth microdilution method. Quality control for the MICs was performed using the reference strains *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603. Carbapenemase production was screened for by the modified Hodge test (5). The presence of bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{NDM-1} genes was determined using previously published methods (6, 7). Moreover, the presence of bla_{IMP}, bla_{VIM}, and bla_{KPC} genes was screened for using previously published primer sets (8) as follows: 2 min of initial denaturation at 94°C, 30 cycles of PCR, each consisting of 30 s at 94°C and 45 s at 68°C, and 3 min of final extension at 72°C. Furthermore, the KPC type was identified using the primers KPC-F1 (5′-ATCGCCGTCTAGTTCTGCTG-3′) and KPC-R1 (5′-CCCTCGAGCGCGAGTCTA-3′), constructed using the KPC-2-producing *K. pneumoniae* sequence (GenBank accession no. AY034847). Additionally, the presence of plasmid-mediated *ampC* genes was investigated by multiplex PCR, as described previously (9). For molecular typing, we used multilocus sequence typing (MLST) (see http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

TABLE 1 MICs of *K. pneumoniae* Kp3018, its transconjugant TcKp3018, and strain *E. coli* C600

Antibiotic(s)[a]	MIC (μg/ml) against:		
	K. pneumoniae Kp3018	*E. coli* TcKp3018	*E. coli* C600
Imipenem	>8	4	≤1
Imipenem + DPA	>8	4	ND[b]
Meropenem	>8	4	≤1
Ceftazidime	>16	16	≤1
Ceftazidime + CLA	>4	>4	ND
Cefotaxime	>32	>32	≤8
Cefotaxime + CLA	4	4	ND
Aztreonam	>16	>16	≤8
Cefpirome	>16	>16	≤8
Cefmetazole	>32	≤4	≤4
Piperacillin-tazobactam	>64	>64	≤8
Gentamicin	>8	>8	≤1
Amikacin	≤4	≤4	≤4
Levofloxacin	>4	≤0.5	≤0.5
TMP-STX	≤2	≤2	≤2
Fosfomycin	>16	≤4	≤4

[a] DPA, dipicolinic acid; CLA, clavulanic acid; TMP-STX, trimethoprim-sulfamethoxazole.
[b] ND, not determined.

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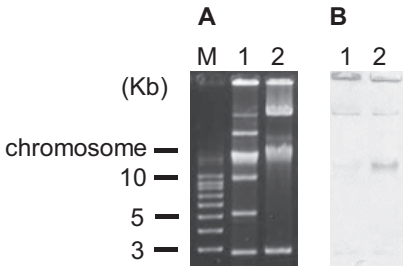

FIG 1 Plasmid profiles (A) and the results of Southern hybridization analysis carried out with a bla_{KPC-2} probe (850 bp) (B). Lane M, molecular weight; lane 1, *K. pneumoniae* strain Kp3018; lane 2, *E. coli* C600 transconjugant strain TcKp3018.

Conjugation experiments were performed in LB broth with strain Kp3018 as a donor and the rifampin-resistant strain *E. coli* C600 as the recipient. Transconjugants were selected on Drigalski agar (bromothymol blue [BTB] agar) plates containing 80 μg/ml rifampin and 1 μg/ml imipenem (10). The MICs of the transconjugants were determined as described above. Plasmid DNA was extracted from donors and transconjugants using a NucleoBond Xtra midi (TaKaRa Bio, Shiga, Japan), according to the manufacturer's instructions, and was then analyzed by electrophoresis on a 0.7% (wt/vol) agarose gel. The size of transferred plasmids was estimated using a OneSTEP ladder supercoiled plasmid (Nippon Gene, Tokyo, Japan). Southern hybridization experiments were performed using a DIG-High Prime DNA labeling and detection starter kit I (Roche Diagnostics, Tokyo, Japan). Probes (850 bp) were generated by PCR using the primers KPC-F1 and KPC-R1. The genetic structures encompassing bla_{KPC} in *K. pneumoniae* Kp3018 and its transconjugant were investigated by PCR mapping and subsequent sequencing, as described previously (11). A partial sequence (2,323 bp) encompassing bla_{KPC} has been deposited in the DDBJ/EMBL/GenBank database under the accession no. AB854326.

In the present study, *K. pneumoniae* strain Kp3018 was found to be resistant to 11 antibiotics, including imipenem and meropenem, but not to amikacin and trimethoprim-sulfamethoxazole, as shown in Table 1. Furthermore, it phenotypically produced carbapenemases and harbored bla_{KPC-2}, $bla_{CTX-M-15}$, and bla_{SHV-11}. In contrast, no other β-lactamase genes of classes A (bla_{TEM}) and B (bla_{IMP}, bla_{VIM}, and bla_{NDM}) were detected. These results are similar to those of previous studies, which demonstrated that KPC-producing isolates are resistant to not only all β-lactam antibiotics, including carbapenems, but also to some non-β-lactam antibiotics, such as aminoglycosides and fluoroquinolones (1, 3). Moreover, Kp3018 was grouped into sequence type 11 (ST11). These KPC-2-producing *K. pneumoniae* isolates of ST11 have been reported from various geographic regions, such as China (12), Greece (13), Poland (14), the United Kingdom (15), and Brazil (16, 17). Isolates producing $bla_{CTX-M-15}$ and bla_{SHV-11} have also been reported in Singapore (18). Furthermore, the coproduction of KPC-2 and CTX-M-15 has been described in *K. pneumoniae* ST437 (clonal complex 11) isolated from patients with bloodstream infections in Brazil (19).

Plasmid analysis detected two plasmids of ca. 50 kb and ca. 190 kb in Kp3018 (Fig. 1A). Among these, the plasmid of ca. 190 kb was transferred successfully to *E. coli* C600 by conjugation. A transconjugant, *E. coli* C600 TcKp3018, showed a carbapenem-resistant phenotype and possessed bla_{KPC-2} in addition to $bla_{CTX-M-15}$. Furthermore, Southern hybridization revealed that bla_{KPC-2} was present on the plasmid of ca. 190 kb in both Kp3018 and TcKp3018 (Fig. 1B). To investigate the sequence of the bla_{KPC-2} genetic environment, PCR mapping was performed using the previously published primers 816U and 4714 (11). The ca. 2.3-kb sequence (IS*Kpn8*-bla_{KPC-2}–IS*Kpn6*-like) encompassing bla_{KPC-2} was analyzed and was found to be identical to that of plasmid pKP048 (GenBank accession no. FJ628167) in a *K. pneumoniae* isolate from China (20), except for a deletion of 11 bp among the 38-bp complete right invert repeat of Tn*3* located between downstream of IS*Kpn8* and upstream of the bla_{KPC-2} gene. A previous study demonstrated that Tn*4401* without IS*Kpn7* is present in KPC-2-producing *K. pneumoniae* isolates of ST11 in Brazil (16). Although we did not investigate the overall structure of the transmissible plasmid in Kp3018, we speculate that IS*Kpn8* was independently inserted downstream of the Tn*3*-based element by transposition. However, in this study, we cannot exclude the possibility that this KPC-2-positive strain of ST11 was acquired outside Brazil, since KPC-producing *K. pneumoniae* isolates with IS*Kpn8* have not yet been reported in Brazil.

In conclusion, we identified a KPC-2-producing *K. pneumoniae* strain, Kp3018, for the first time in Japan and demonstrated that diversity of the bla_{KPC-2} genetic environment within the ca. 190-kb transferable plasmid was conferred by Tn*3*-based transposition. Since the dissemination of *Enterobacteriaceae* possessing carbapenemase activities, including KPC, pose a significant threat to the management of those infections worldwide, it will be important to continuously monitor the prevalence of carbapenemase-producing *Enterobacteriaceae* in Japan.

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