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Emergence in Japan of an isolate of *Klebsiella pneumoniae* co-harbouring bla_{KPC-2} and *rmtB*

Hiroki Uchida[a], Tatsuya Tada[a,*], Mari Tohya[a], Yoshie Sugahara[b], Akira Kato[c], Isao Miyairi[d,e], Teruo Kirikae[a]

[a] Department of Microbiology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
[b] Department of Nursing, National Center for Child Health and Development, Setagaya-ku, Tokyo 157-8535, Japan
[c] Clinical Microbiology Laboratory, Department of Clinical Laboratory Medicine, National Center for Child Health and Development, Setagaya-ku, Tokyo 157-8535, Japan
[d] Division of Infectious Diseases, Department of Medical Subspecialties, National Center for Child Health and Development, Setagaya-ku, Tokyo 157-8535, Japan
[e] Department of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN 38138, USA

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ABSTRACT

Objectives: Here we describe a clinical isolate of *Klebsiella pneumoniae* ST11 harbouring both bla_{KPC-2} and *rmtB* genes in Japan.
Methods: A carbapenem- and aminoglycoside-resistant *K. pneumoniae* was isolated from an inpatient in Japan. Whole-genome sequencing (WGS) was performed using an Illumina next-generation sequencer.
Results: Minimum inhibitory concentrations (MICs) of meropenem and amikacin were ≥512 μg/mL. WGS analysis revealed that the isolate harboured both bla_{KPC-2} and *rmtB*. The genetic environments of bla_{KPC-2} and *rmtB* consisted of IS*6*–*orfA*–*orfB*–IS*481*–bla_{KPC-2}–IS*Kpn6*–*korC*–*orfC*–*orfD*–*rep*–*tnp*–Tn*3*–IS*6* and IS*6*–IS*91*–*orfE*–*orfF*–bla_{TEM-1}–*rmtB*–*orfG*–IS*6*, respectively. These genetic environments are similar (>99% identity) to those of *K. pneumoniae* WCHKP040035 (accession no. **CP028796**) isolated in a Chinese hospital. The bla_{KPC-2} and *rmtB* genes were located on a 130-kb IncFII plasmid.
Conclusions: This is the first report of a *K. pneumoniae* clinical isolate from Japan co-harbouring bla_{KPC-2} and *rmtB* on a 130-kb plasmid.

1. Introduction

The emergence of carbapenem-resistant Enterobacteriaceae is a serious health threat worldwide [1]. *Klebsiella pneumoniae* carbapenemase (KPC) enzymes are class A serine β-lactamases that hydrolyse β-lactam antimicrobials, including carbapenems, that were first reported in the USA in 2001 [2]. Bloodstream infections caused by KPC-producing isolates have high mortality rates as the remaining treatment options, including colistin and tigecycline, are limited [3]. KPC-producing Gram-negative bacteria have been reported worldwide [4], with KPC-1 first reported in *K. pneumoniae* isolated from patients in the USA [2].

Gram-negative pathogens producing 16S rRNA methylases are also of worldwide concern because they are extremely resistant to all clinically important aminoglycosides, including gentamicin, tobramycin and amikacin [5]. To date, ten 16S rRNA methylases (ArmA, RmtA, RmtB, RmtC, RmtD, RmtE, RmtF, RmtG, RmtH and NpmA) have been described [6], with RmtB first reported in a strain of *Serratia marcescens* from Japan in 2004 [7]. Up to now, there have been three reports on *K. pneumoniae* producing 16S rRNA methylases in Japan, including ArmA-producers isolated in 2004 and 2017 [8,9] and RmtB-producers isolated in 2001 [10].

This study describes a clinical isolate of *K. pneumoniae* from Japan co-harbouring bla_{KPC-2} and *rmtB* genes.

2. Materials and methods

Klebsiella pneumoniae strain NCCHD1261-1 was isolated from a rectal swab sample obtained from a 3-year-old boy. The patient was directly referred from a hospital in China to a tertiary children's hospital in Japan in 2017 for evaluation of an undiagnosed liver disease. He had a history of recurrent urinary tract infection and had been treated with cephalosporins in China. The patient was screened by active surveillance for antimicrobial-resistant pathogens before hospitalisation in Japan. Minimum inhibitory concentrations (MICs) of antimicrobial agents

* Corresponding author.
E-mail address: t-tada@juntendo.ac.jp (T. Tada).

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were determined by the microdilution method according to the 2017 guidelines of the Clinical and Laboratory Standards Institute (CLSI M100-S27). Antimicrobial susceptibility testing was performed in triplicate. The whole genome of the isolate was extracted and was sequenced using a MiSeq Next-Generation Sequencer (Illumina Inc., San Diego, CA) and analysed using CLC Genomics Workbench v.8.0 (CLC bio, Tokyo, Japan) as described previously [11]. Antimicrobial resistance and virulence genes were identified using ResFinder 3.0 (https://cge.cbs.dtu.dk/services/ResFinder/) and VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/), respectively. Multilocus sequence typing (MLST) was performed using MLST 1.8 (https://cge.cbs.dtu.dk/services/MLST-1.8/) and was confirmed by PCR-based sequencing as described at the Institut Pasteur (http://bigsdb.pasteur.fr/klebsiella/). To determine the size of the plasmid harbouring bla_{KPC-2} and *rmtB*, a DNA plug, digested with S1 nuclease, was separated by pulsed-field gel electrophoresis (PFGE) and was subjected to Southern hybridisation as described previously [11]. Signal detection was performed using a DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science, Indianapolis, IN).

3. Results

Antimicrobial susceptibility testing showed that *K. pneumoniae* NCCHD1261-1 was resistant to amikacin, ampicillin, ampicillin/sulbactam, arbekacin, aztreonam, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem and penicillin G but was susceptible to colistin (Table 1). The isolate harboured bla_{TEM-1}, $bla_{SHV-115}$, bla_{KPC-2}, $bla_{CTX-M-65}$, *rmtB* and *aadA2* genes. No other known carbapenemase-encoding genes were detected. The quinolone resistance-determining region of the isolate contained three mutations with amino acid substitutions, including two (S83I and D87E) in GyrA and one (S80I) in ParC [12]. Up to now, 30 quinolone-resistant *K. pneumoniae* isolates with the same amino acid mutations in GyrA and ParC have been registered in GenBank (https://www.ncbi.nlm.nih.gov/genbank/), of which 27 were from China, 2 from the USA and 1 from Japan.

Table 1
Minimum inhibitory concentrations (MICs) of antimicrobial agents for *Klebsiella pneumoniae* NCCHD1261-1.

Antimicrobial agent	MIC (μg/mL)
Amikacin	>512
Ampicillin	>512
Ampicillin/sulbactam	>512
Arbekacin	>512
Aztreonam	>512
Cefotaxime	>512
Ceftazidime	>512
Ciprofloxacin	64
Colistin	0.25
Gentamicin	>512
Imipenem	64
Levofloxacin	32
Meropenem	512
Penicillin G	>512
Tigecycline	1

Klebsiella pneumoniae NCCHD1261-1 belongs to sequence type (ST) 11. To date, 247 isolates belonging to ST11 from various countries have been registered in the MLST database (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Twelve of these isolates produce KPC-2, including three from Argentina, seven from Brazil and one each from Poland and the USA, but there is no information on aminoglycoside resistance factors. *Klebsiella pneumoniae* NCCHD1261-1 also harboured the virulence factor gene *ccl*.

Southern hybridisation showed that both bla_{KPC-2} and *rmtB* were located on a 130-kb plasmid (data not shown) belonging to the incompatibility group IncFII.

Mating-out assays were performed as described previously [13] and revealed that the conjugation frequency of the plasmid to rifampicin-resistant *Escherichia coli* DH5α was 2.2×10^{-8}.

The genetic environments surrounding bla_{KPC-2} and *rmtB* were derived from their contig data following assembly of the raw read data (Fig. 1). The genetic environment of bla_{KPC-2} consisted of IS*6*–*orfA*–*orfB*–IS*481*–bla_{KPC-2}–IS*Kpn6*–*korC*–*orfC*–*orfD*–*rep*–*tnp*–Tn*3*–IS*6*, with *orfA* encoding an adenine-specific DNA methyltransferase, *orfB* encoding a recombinase, *orfC* encoding an antirestriction protein and *orfD* encoding a hypothetical protein. The genetic environment of *rmtB* consisted of IS*6*–IS*91*–*orfE*–*orfF*–bla_{TEM-1}–*rmtB*–*orfG*–IS*6*, with *orfE* encoding a hypothetical protein, *orfF* encoding a recombinase and *orfG* encoding a sodium/proton antiporter. These genetic environments are similar (>99% identity) to those of plasmid pKPC-2 in *K. pneumoniae* WCHKP040035 (accession no. **CP028796**) obtained in a hospital in China.

Klebsiella pneumoniae WCHKP040035 belongs to ST11, and plasmid pKPC-2 belongs to the incompatibility group IncFII.

4. Discussion

To our knowledge, this is the first report of a Gram-negative bacterium from Japan co-harbouring bla_{KPC-2} and *rmtB*. To date, there have been 13 reports of *K. pneumoniae* isolates co-harbouring bla_{KPC-2} and *rmtB*, including 9 from China [14–22] and 1 each from India, Greece, Brazil and South Korea [23–26]. In Japan, KPC-2-producing *K. pneumoniae* was first isolated from a patient treated at a Brazilian hospital [27] belonging to ST11.

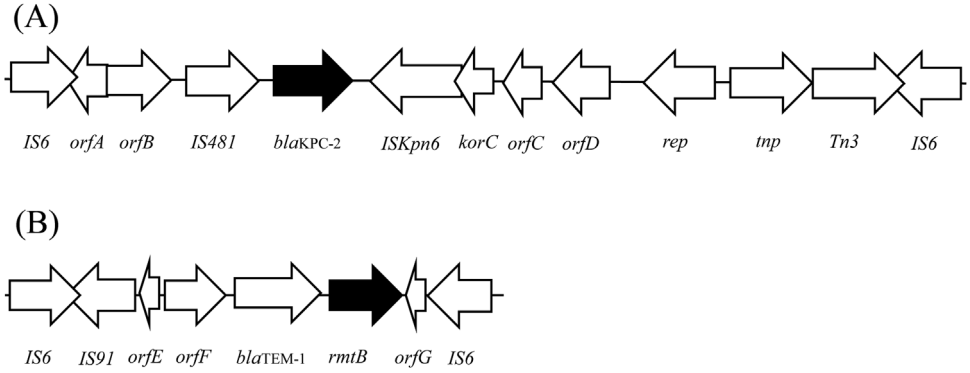

Fig. 1. Genetic environments surrounding (A) bla_{KPC-2} (accession no. **LC423559**) and (B) *rmtB* (accession no. **LC424160**) in *Klebsiella pneumoniae* NCCHD1261-1. *orfA*, adenine-specific DNA methyltransferase gene; *orfB*, recombinase gene; *orfC*, antirestriction protein gene; *orfD* and *orfE*, hypothetical protein genes; *orfF*, recombinase gene; and *orfG*, sodium/proton antiporter gene.

Klebsiella pneumoniae ST11 is an internationally distributed high-risk multidrug-resistant clone carrying carbapenem resistance genes, including bla_{KPC}, bla_{NDM} and bla_{OXA-48}, as well as aminoglycoside resistance genes, including *armA* and *rmtB*. ST11, which belongs to clonal complex (CC) 258, is the major ST among *K. pneumoniae* producing KPC enzymes in Asian and Latin American countries, NDM enzymes in the Czech Republic, Switzerland, Thailand, Australia, the USA, the United Arab Emirates and Greece, and OXA-48 in Spain [28]. Other STs also belonging to CC258 *K. pneumoniae* producing KPC have been reported in Colombia (ST512), Italy (ST512), Israel (ST512), Spain (ST512), Brazil (ST340) and Greece (ST340) [28].

This is the first report of *K. pneumoniae* co-harbouring bla_{KPC-2} and *rmtB* in Japan. Strain NCCHD1261-1 may have arisen in China as it was isolated from a patient treated initially in China. This study suggests the possible spread in Japan of *K. pneumoniae* carrying carbapenem and aminoglycoside resistance genes on a plasmid, because KPC-producing ST11 *K. pneumoniae* was demonstrated as a predominant clone in China [29]. It is therefore necessary to survey carbapenem- and aminoglycoside-resistant Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, obtained in medical settings throughout Japan and to monitor import of these pathogens into Japan from overseas.

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Competing interests

None declared.

Ethical approval

This study was approved by the Biosafety Committee of Juntendo University (Tokyo, Japan) [approval no. BSL2/29-1].

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