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EPIDEMIOLOGY AND SURVEILLANCE

Genomic Characterization of Carbapenemase-Producing *Klebsiella pneumoniae* with Chromosomally Carried bla_{NDM-1}

Noriko Sakamoto,[a] Yukihiro Akeda,[a,b,c] Yo Sugawara,[a] Dan Takeuchi,[a] Daisuke Motooka,[d] Norihisa Yamamoto,[a,b,c] Warawut Laolerd,[e] Pitak Santanirand,[e] Shigeyuki Hamada[a]

[a]Japan-Thailand Research Collaboration Center for Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

[b]Division of Infection Control and Prevention, Osaka University Hospital, Osaka University, Osaka, Japan

[c]Department of Infection Control and Prevention, Graduate School of Medicine, Osaka University, Osaka, Japan

[d]Department of Infection Metagenomics, Genome Information Center, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

[e]Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

ABSTRACT We report here *Klebsiella pneumoniae* strains carrying chromosomal bla_{NDM-1} in Thailand. The genomes of these two isolates include a 160-kbp insertion containing bla_{NDM-1}, which is almost identical to that in the IncHI1B-like plasmid. Further analysis indicated that IS*5*-mediated intermolecular transposition and Tn*3* transposase-mediated homologous recombination resulted in the integration of bla_{NDM-1} into the chromosome from an IncHI1B-like plasmid. The spread of this type of carbapenem-resistant *Enterobacteriaceae* may threaten public health and warrants further monitoring.

KEYWORDS IS*5*, *Klebsiella pneumoniae*, Tn*3*, beta-lactamases, bla_{NDM}, carbapenemase-producing *Enterobacteriaceae*, carbapenems

Carbapenems are the antibiotics of last resort for combating multidrug-resistant organisms. However, their efficacy is increasingly compromised by carbapenem-resistant *Enterobacteriaceae* (CRE), which produce a variety of carbapenemases, including New Delhi metallo-β-lactamase (NDM). NDM-1 was initially reported in 2009 (1), and NDM-1-producing CRE originating on the Indian subcontinent have rapidly disseminated into different parts of the world (2). Carbapenemase genes, including bla_{NDM-1}, are typically plasmid borne and are responsible for the spread of CRE by horizontal gene transfer. Plasmids act as the scaffolds upon which arrays of antibiotic resistance genes are assembled, generating multiple-drug-resistant phenotypes in *Enterobacteriaceae*. However, only a limited number of CRE have been found to carry carbapenemase genes on their chromosomes (3–6). Notably, only two strains of *Escherichia coli* have been reported to carry chromosomally integrated bla_{NDM-1} in China and India (7, 8). However, no such organisms have been found among *Klebsiella pneumoniae* strains.

We have previously used long- and short-read whole-genome sequencing (WGS) by the PacBio RSII and Illumina MiSeq systems to examine clinical isolates of *K. pneumoniae* in a molecular epidemiology study of CRE in Bangkok, Thailand (Y. Akeda, W. Laolerd, Y. Sugawara, N. Sakamoto, D. Motooka, N. Yamamoto, D. Takeuchi, R. K. Shanmugakani, I. Nishi, M. Suzuki, K. Shibayama, P. Santanirand, K. Tomono, S. Hamada, submitted for publication) (see the supplemental material). The long reads acquired from PacBio RSII were assembled using the PacBio hierarchical genome assembly process (version 3) through the SMRT portal. To further improve the quality of the assembled sequences, short-read mapping was performed using Illumina MiSeq read data from CLC Workbench version 7.5 (CLC bio). During the course of that study, we noted that two *K. pneumoniae* isolates (strains KP64 and KP67) collected from different patients did not

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Address correspondence to Yukihiro Akeda, akeda@biken.osaka-u.ac.jp.

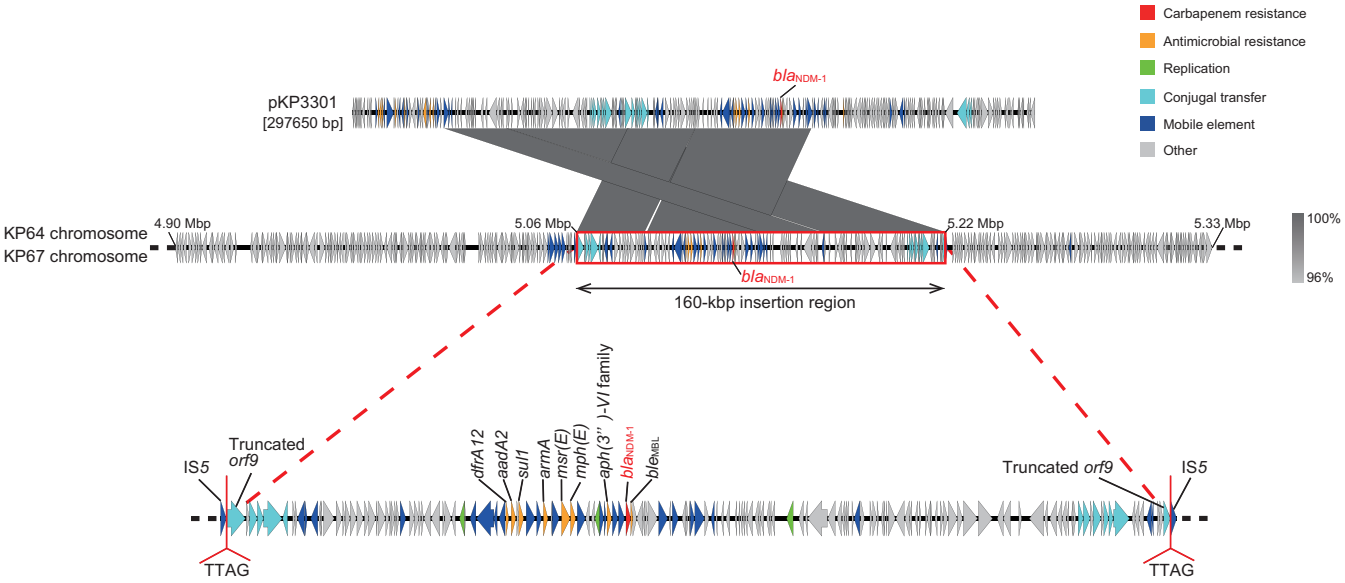

FIG 1 Comparison of chromosomal 160-kbp regions containing bla_{NDM-1} in strains KP64 and KP67 and bla_{NDM-1}-carrying plasmid pKP3301 from strain KP33. The gray-shaded area indicates the regions with high identity (>99% similarity) between the two sequences. The 160-kbp region containing bla_{NDM-1} is highlighted with a red box. Nucleotide letters below the sequences of the KP64 and KP67 chromosomes represent target site duplications.

carry the bla_{NDM-1} gene on their plasmids. To determine the location of bla_{NDM-1} in these strains, we performed Southern blotting using probes for bla_{NDM-1} and 16S rRNA genes after pulsed-field gel electrophoresis (PFGE) (see Fig. S1A and B in the supplemental material). A bacterial conjugation assay with strains KP64, KP67, and KP33 (a control strain carrying the bla_{NDM-1} gene on its plasmid), with the sodium azide-resistant *E. coli* strain TUM3456, was used to detect transconjugants. However, no bla_{NDM-1}-carrying transconjugants were obtained from KP64 and KP67, whereas KP33 produced transconjugants. These results suggest that bla_{NDM-1} in strains KP64 and KP67 is not carried on a plasmid, but strains KP64 and KP67 carry the bla_{NDM-1} gene on their chromosomes.

The genomic characteristics of strains KP64, KP67, and KP33, all of which belong to multilocus sequence type (MLST) ST14, are summarized in Table 1. The chromosomal genomic sequences for KP64 and KP67 are closely related to that of KP33, but three large insertions were observed (Fig. S2). Two insertion sequences (67-kbp and 105-kbp insertion regions) harbor conjugal transfer systems and ABC transporter genes, respectively. However, no significant similarities with plasmids, mobile elements, or target site duplications were observed. A database search with the WGS data for strains KP64 and KP67 revealed that these isolates carry bla_{NDM-1} on their chromosomes, in addition to diverse antimicrobial resistance genes (160-kbp insertion region). Both the KP64 and KP67 strains contain two plasmids, plasmids pKP6401 and pKP6402 and plasmids pKP6701 and pKP6702, respectively. pKP6401 and pKP6701 contain the genes associated with plasmid partitioning and type I restriction-modification systems but no antimicrobial resistance genes. They share more than 99% sequence identity with the 104-kbp plasmid pPKPN2 from NDM-1-producing *K. pneumoniae* isolate PittNDM01 (9). pKP6402 and pKP6702 each contain five antimicrobial resistance genes directed against β-lactams and aminoglycosides. Approximately 45% of the pPKPN3 sequence shares ~99% identity with pKP6402 and pKP6702. pKP6402 and pKP6702 lack several antimicrobial resistance genes directed against β-lactams, aminoglycosides, sulfonamide, and rifampin that are carried by pPKPN3. An antimicrobial susceptibility test showed that strains KP64 and KP67 are multidrug resistant, including to carbapenems.

WGS revealed that the chromosomes of strains KP64 and KP67 include a 160-kbp region that contains bla_{NDM-1} and seven other antimicrobial resistance genes (Fig. 1 and

TABLE 1 Genomic features of clinical isolates of *K. pneumoniae* containing the bla_{NDM-1} gene

Isolate	Genomic structure	Length (bp)	GC content (%)	No. of CDSs[a]	Resistant genes	Replicon type	DDBJ accession no.
KP64	Chromosome	5,587,050	57.06	5,405	bla_{NDM-1}, *aadA2*, *armA*, *aph(3′)-VIa*, *msr*(E), *mph*(E), *sul1*, *dfrA12*, bla_{SHV-28}, $bla_{CTX-M-15}$, bla_{OXA-1}, *cat*, *aac(6′)Ib-cr*, *fosA*, *oqxAB*, *dfrA1*		AP018750
	Plasmid pKP6401	102,903	50.99	137	Not detected	FIB	AP018751
	Plasmid pKP6402	31,835	52.66	49	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1A}, *aac(6′)Ib*, *aadA2*	R	AP018752
KP67	Chromosome	5,587,048	57.06	5,400	bla_{NDM-1}, *aadA2*, *armA*, *aph(3′)-VIa*, *msr*(E), *mph*(E), *sul1*, *dfrA12*, bla_{SHV-28}, $bla_{CTX-M-15}$, bla_{OXA-1}, *cat*, *aac(6′)Ib-cr*, *fosA*, *oqxAB*, *dfrA1*		AP018753
	Plasmid pKP6701	102,917	50.99	131	Not detected	FIB	AP018754
	Plasmid pKP6702	31,833	52.65	48	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1A}, *aac(6′)Ib*, *aadA2*	R	AP018755
KP33	Chromosome	5,256,831	57.39	5,137	bla_{SHV-28}, $bla_{CTX-M-15}$, bla_{OXA-1}, *fosA*, *oqxAB*, *cat*, *aac(6′)Ib-cr*, *dfrA1*		AP018747
	Plasmid pKP3301	296,750	46.54	404	bla_{NDM-1}, *aadA2*, *armA*, *aph(3′)-VIa*, *msr*(E), *mph*(E), *sul1*, *dfrA12*, $bla_{CTX-M-15}$, *aac-(6′)-Ib*, *aac(6′)Ib-cr*, *cat*, *dfrA1*, *dfrA14*, *qnrB1*, *tet*(D)	HI1B-like	AP018748
	Plasmid pKP3302	102,910	50.98	132	Not detected	FIB	AP018749

[a]CDSs, coding DNA sequences.

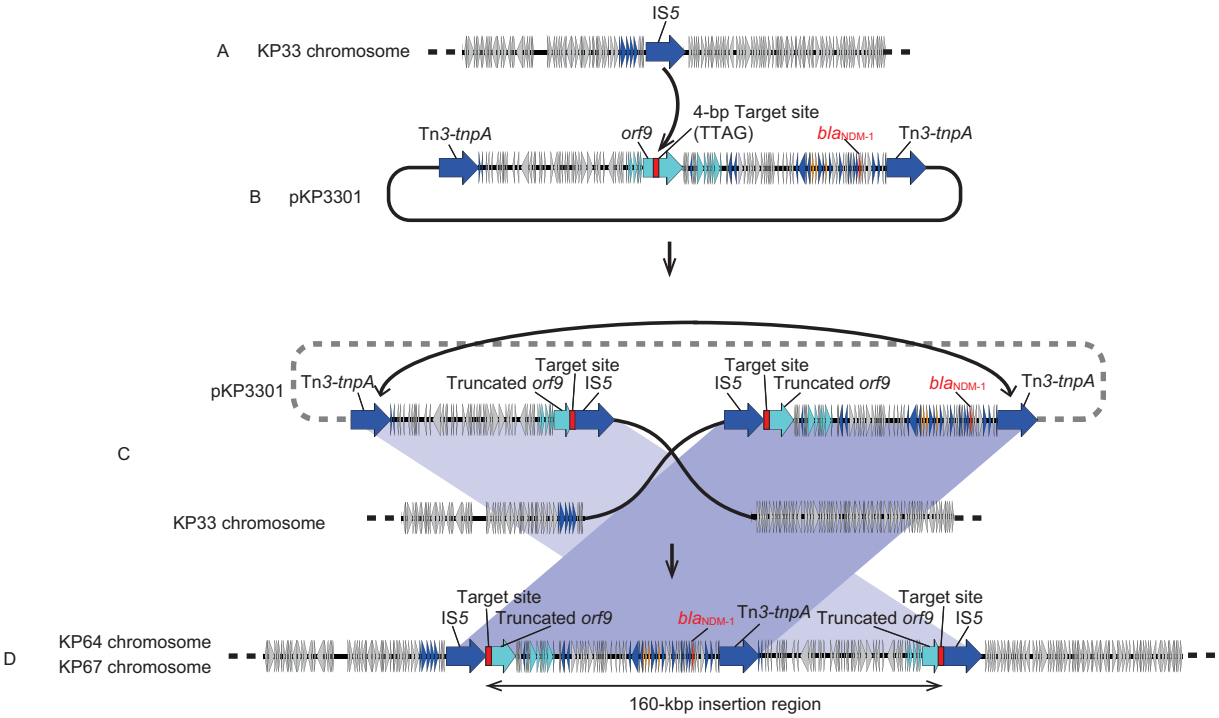

FIG 2 Hypothetical mechanism of integration of the 160-kbp region of plasmid pKP3301 into the KP64 and KP67 chromosomes. (A) Schematic view of the chromosome of strain KP33 carrying an IS*5* copy at the putative insertion site in the 160-kbp region. (B) Plasmid pKP3301 containing bla_{NDM-1}, *orf9*, and a 4-bp target site (AATG) in the middle of the *orf9* gene. (C) IS*5* on the chromosome of strain KP33 recognizes the 4-bp target site in *orf9*, intermolecular transposition generates a cointegrate molecule, and the IncHI1B-like plasmid moiety is truncated (dashed line) by Tn*3* transposase-mediated homologous recombination. (D) Generation of the 160-kbp region bracketed by IS*5* copies. Purple boxes represent homologous regions among different strains.

Fig. S2). This region shares >99% sequence identity with the 297-kbp IncHI1B-like bla_{NDM-1}-carying plasmid pKP3301 from strain KP33, which belongs to ST14 (Table 1 and Fig. S3). pKP3301 is closely related to the sequences of the plasmids from the NDM-1-producing *K. pneumoniae* isolates PittNDM01 and KP617, which originated in India and South Korea, respectively (9, 10), suggesting that ST14 carrying an IncHI1B-like plasmid is the putative ancestor of strains KP64 and KP67.

Further WGS analysis revealed that IS*5* copies were located at both ends of this 160-kbp region and that these two copies share >99% similarity with the IS*Kpn26* family of transposases. We also found duplicated TTAG target sites for IS*5* and truncated *orf9* genes adjacent to the IS*5* copies (Fig. 1). Based on this information, we hypothesized that the following mechanism is responsible for the insertion of the genomic locus containing bla_{NDM-1} into isolates KP64 and KP67 from a plasmid carrying bla_{NDM-1} (Fig. 2). An IS*5* copy is also situated at the insertion site of the 160-kbp region on the chromosome of strain KP33 (Fig. 2A), whereas pKP3301 carries the 160-kbp region bracketed by Tn*3* transposase copies (Fig. 2B). It is noteworthy that both the KP64 and KP67 strains carry a copy of the Tn*3* transposase in the middle of the 160-kbp insertion region. IS*5* on the chromosome may then have recognized the 4-bp target site in the *orf9* gene in pKP3301, causing the intermolecular transposition that generated the cointegrant (11) (Fig. 2A to C), followed by homologous recombination between the two Tn*3* transposase copies (Fig. 2C and D).

There have been several reports of *Enterobacteriaceae* carrying bla_{NDM-1} on their chromosomes, although only two cases have been reported for *E. coli*, in China (7) and India (8), but none have been reported for *K. pneumoniae*. A WGS analysis of Chinese *E. coli* strain Y5 showed that bla_{NDM-1} was embedded in its chromosome, and an IS*CR1* element probably mediated the transposition of bla_{NDM-1} from a plasmid possibly derived from *Proteus mirabilis* PM58. Therefore, the integration

mechanism differs completely from that involved in the two strains analyzed in this study. Poirel et al. previously suggested the chromosomal location of bla_{NDM-1} in *E. coli*, based on the results of a conjugation assay. However, the integration mechanism and genetic context surrounding this chromosomal bla_{NDM-1} gene are unclear (8). The bla_{OXA-48}-type and bla_{KPC} genes are carried on certain transposons, such as Tn*1999* and Tn*4401*, and are sometimes integrated into chromosomes by these transposons; however, bla_{NDM} was originally carried on transposon Tn*125* in an *Acinetobacter* species. The Tn*125* structure and its flanking sequences in the *Enterobacteriaceae* are frequently inserted by various mobile elements, generating diverse genetic contexts for bla_{NDM} (12, 13). The plasticity of the genetic contexts for bla_{NDM} indicates that this gene can be transferred by various mechanisms from plasmids to chromosomes, making tracing the origins of chromosomal bla_{NDM} genes very complicated.

In conclusion, this is the first report of *K. pneumoniae* strains carrying the bla_{NDM-1} gene on their chromosomes. We attribute the genetic transmission of bla_{NDM-1} from the IncHI1B-like plasmid to the chromosomes of these strains to IS*5* and the Tn*3* transposase. The bla_{NDM} genes are potentially transferable between plasmid and chromosome via multiple mobile elements; hence, monitoring the chromosomal bla_{NDM} genes should be epidemiologically important.

Accession number(s). The WGS data are available from the DDBJ (DNA Data Bank of Japan) database under accession numbers AP018747, AP018748, AP018749, AP018750, AP018751, AP018752, AP018753, AP018754, and AP018755.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01520-18.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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We declare no conflict of interest.

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