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Genetic Environment of the bla_{KPC-2} Gene in a *Klebsiella pneumoniae* Isolate That May Have Been Imported to Russia from Southeast Asia

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ABSTRACT The nucleotide sequence of a bla_{KPC-2}-harboring plasmid (pKPCAPSS) from *Klebsiella pneumoniae* ST273 isolated in Saint Petersburg, Russia, from a patient with history of recent travel to Vietnam is presented. This 127,970-bp plasmid possessed both IncFII and IncR replicons. bla_{KPC-2} was localized on a hypothetical mobile element. This element was flanked by 38-bp inverted Tn*3* repeats and included a Tn*3*-specific transposase gene, macrolide resistance operon (*mphA-mrx-mphR*), and a fragment of bla_{TEM} with unique polymorphisms.

KEYWORDS KPC-2, *Klebsiella pneumoniae*, IncR, IncFII, transposons

Klebsiella pneumoniae, which produces *K. pneumoniae*-type carbapenemases (KPCs), is of great concern to health service professionals worldwide. Global dissemination of KPCs is mostly attributed to expansion of successful clones and to some extent to horizontal transfer of mobile genetic elements. KPCs can be found in a growing number of different *K. pneumoniae* sequence types (STs), but clonal group 258 (CG258), which comprises hybrid lineage ST258/ST512 (1) and related variants (2), is predominant. Commonly KPC genes are located on Tn*3*-based transposon Tn*4401*, which has several isoforms (3). These genes are also detected in diverse elements lacking the entire Tn*4401* transposon. These non-Tn*4401* elements (NTE_{KPC}) are divided into three types (4). The bla_{KPC}-bearing elements have been found on plasmids of different lengths in different incompatibility (Inc) groups (4). The complete genome sequencing of a bla_{KPC-2}-bearing plasmid from a *K. pneumoniae* ST273 isolate recovered from Russia is presented here.

K. pneumoniae strain KP565 was isolated in 2011 from the urine sample of a female patient with a pelvic infection who had been hospitalized due to malignancy. The patient had a history of recent travel to Vietnam. The isolate was tested according to CLSI recommendations (5) and demonstrated resistance to β-lactams (ampicillin, cefotaxime, ceftazidime, cefepime, aztreonam, ertapenem, imipenem, meropenem, and biapenem MICs, ≥64 μg/ml), gentamicin (MIC, 126 μg/ml), fosfomycin (MIC, 128 μg/ml), ciprofloxacin (MIC, >256 μg/ml), co-trimoxazole (MIC, >32 μg/ml), chloramphenicol (MIC, 256 μg/ml), and polymyxin B (MIC, 16 μg/ml), but it was susceptible to amikacin (MIC, 1 μg/ml), and tigecycline demonstrated a MIC of 0.25 μg/ml. PCR amplification and sequencing of the amplicons revealed the presence of bla_{KPC-2}, and multilocus sequence typing (MLST) demonstrated that the isolate belonged to the

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ST273 lineage (6). This was the first isolation of KPC-producing *K. pneumoniae* ST273. Recently KPC-positive ST273 has also been reported in Italy (7).

Carbapenem resistance transfer experiments were performed by filter mating of KP565 as a donor and *Escherichia coli* C-600 Az^r Rif^r as a recipient. Transconjugants were selected on Muller-Hinton agar with meropenem (0.5 μg/ml) and sodium azide (300 μg/ml). The presence of plasmids of the same size in the donor (clinical *K. pneumoniae* isolate KP565) and *E. coli* C600 transconjugant was demonstrated by electrophoresis (see Fig. S1 in the supplemental material). Plasmid DNA was purified from the *E. coli* C600 transconjugant using the GeneJET plasmid miniprep kit (ThermoFisher Scientific). Plasmid sequencing was performed on an Ion Torrent PGM platform with average coverage of 117. The sequencing reads were *de novo* assembled into consensus contigs using Newbler v.2.7 (Roche Diagnostics, Switzerland). The gaps between *de novo*-assembled contigs were filled using Sanger sequencing. The resulting circular DNA was 127,970 bp in length, with an average GC content of 51.7%. The 134 potential open reading frames (ORFs) were predicted and annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

PlasmidFinder (https://cge.cbs.dtu.dk//services/PlasmidFinder/) facilitated the identification of an IncR replicon (bp 18474 to 18742) (8). Furthermore, the annotation of the pKPCAPSS sequence also indicated the presence of a rare IncFII-like replicon (bp 77525 to 73388) not yet included in the PlasmidFinder database. Mosaic IncR/IncFII-like plasmids carrying KPC genes have already been reported in China. Comparison of pKPCAPSS, mosaic plasmid pKP048 as well as of related plasmids pE66An and pKF3-94 is presented in Fig. S2 in the supplemental material. Plasmid pKP048 (GenBank accession no. FJ628167.2) demonstrated 51% query coverage and 98% similarity (9). The IncR and IncFII replicons of pKP048 and pKPCAPSS were identical, but their relative locations were significantly different. Both plasmids harbor bla_{KPC} in similar genetic environments, and pKP048 additionally harbors bla_{DHA-1}. The highest similarity scores (50% query coverage, 99% identity) were obtained with the corresponding parts of the IncR plasmid pKF3-94 (GenBank accession no. FJ876826.1) from *K. pneumoniae* recovered from China. The plasmid pKF3-94 harbored two β-lactamase genes, $bla_{CTX-M15}$ and bla_{TEM-1B} (10), absent in plasmid pKPCAPSS. An IncFII-like replicon has already been identified in plasmid pE66An from *Escherichia coli* isolated in Vietnam (accession no. NC_020086). pE66An and pKPCAPSS plasmids demonstrated 100% nucleotide identity only in the region comprising the FII replicon and a vestigial portion of the transfer locus encoding the FinO, TraX, and TraI proteins. It is therefore plausible that pKPCAPSS emerged through a mosaic fusion of two episomes, one carrying the IncFII replicon and another carrying the IncR replicon. It was impossible to determine whether bla_{KPC-2} originated from one of donor plasmids or was acquired later.

Analysis of the genetic environment of the bla_{KPC-2} gene revealed the presence of a new 17,003-bp potentially mobile element. The structures of this element, the most closely related genetic elements, and transposon Tn*3* are presented in Fig. 1. The hypothetical element was a Tn*3* transposon (including *tnp*A, *tnp*R, and bla_{TEM-1} genes) with the insertion of complex genetic construct. Inverted 38-bp Tn*3* repeats were present on both sides of the mobile elements, which indicate possible transposition activity. The regions of high similarity between pKPCAPSS, pKPC3_SZ (11) and pKP048 (12) can be detected. The presence of Tn*3*-specific transposase (*tnpA*) was common to all plasmids. The second region of high similarity included a remnant of Tn*4401* (bla_{KPC} and partial IS*Kpn6* genes) with genes encoding potential antirestriction and replication proteins and was located upstream of the *tnpR* and bla_{TEM-1} gene complex specific to pKPCAPSS and the Tn*3* transposon. Elements from pKPCAPSS and pKP048 harbored bla_{KPC-2}, and the element from pKPC3_SZ harbored bla_{KPC-3}. A noncoding region (NCR_{211}) and a truncated bla_{TEM} fragment (Δ_{TEM}) were located upstream of bla_{KPC} in elements from the pKPCAPSS and pKPC3_SZ plasmids and were lacking in pKP048. The presence of Δ_{TEM} is used in current nomenclature as a marker of NTE_{KPC} elements type II (NTE_{KPC}-II) (4). The structures of Δ_{TEM} and NCR_{211} were used to further examine the NTE_{KPC}-II elements and describe the three subtypes (4). It is necessary to stress that the

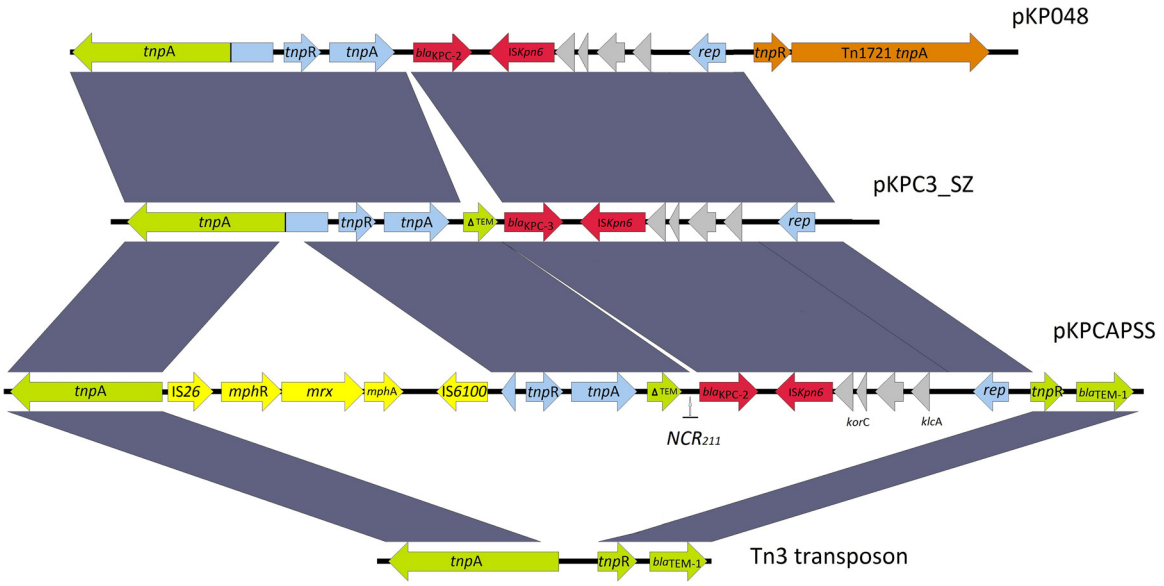

FIG 1 Genetic map and comparison of the hypothetical mobile element from plasmid pKPCAPSS with the bla_{KPC-2} gene environment from plasmids, pKPC3_SZ (11) and pKP048 (9) and with the Tn*3* transposon (23). The hypothetical mobile element was flanked by inverted Tn*3* repeats with the following gene order: Tn*3*-specific transposase gene (green), macrolide resistance operon genes *mphA*, *mrx*, and *mphR* (yellow), *tnpR* and *tnpA* genes identical to those of pKPC3_SZ and pKP048 (blue), insertion of the Δ_{TEM-1} fragment with SNPs (green), noncoding region (NCR_{211}), fragment identical to Tn*4401* that contained the bla_{KPC-2} gene and IS*Kpn6* (red), genes encoding antirestriction proteins (gray), and a gene encoding a replication protein (blue). The part of the Tn*3* transposon containing the *tnp*R and bla_{TEM-1} genes flanked this construction (green).

structure of NTE_{KPC} elements is secondary to the structure of Tn*4401*. In all elements, similar *tnpR* and *tnpA* genes were detected downstream of Δ_{TEM}: their origin at present is unknown. An important feature of the new element was the presence of a macrolide resistance operon flanked by a copy of IS*26* at one end and IS*6100* at the other. Both insertion sequences (ISs) carried transposase genes and had almost identical 14-bp IR elements, so this operon together with IS*26* and IS*6100* may form a composite transposon (13). This gene cluster was earlier found in various genetic locations (14–16), and its dissemination may occur not only through replicative transposition, but also through IS*26*-mediated homologous recombination.

Using BLAST, a number of Δ_{TEM}-bla_{KPC} fragments similar to the fragment from pKPCAPSS were detected in the GenBank database (Table 1). In the majority of these

TABLE 1 Comparison of Δ_{TEM} KPC fragments from the GenBank database

Country of isolation	Species	Δ_{TEM} variant[a]		Intergenic region deletion (bp)[b]	KPC type	GenBank accession no. (reference)
		Deletion (bp)	SNP(s)			
China	*Escherichia coli*	291	643A→G, 549A→G		bla_{KPC-3}	FJ609231.1 (18)
China	*Pseudomonas aeruginosa*	291	643G→A	15	bla_{KPC-2}	KU578314.1 (19)[c]
China	*Klebsiella oxytoca*	291	643G→A		bla_{KPC-2}	HQ651092.1 (20)
China	*Aeromonas hydrophila*	291	643G→A		bla_{KPC-2}	KR014106.1[c]
Chile	*K. oxytoca*	291	643G→A		bla_{KPC-2}	KR052102.1
Chile	*K. pneumoniae*	291	643G→A		bla_{KPC-2}	KR052101.1
Chile	*E. coli*	291	643G→A		bla_{KPC-2}	KR052097.1
China	*A. hydrophila*	291	643G→A		bla_{KPC-2}	KC355363.1
Argentina	*Citrobacter freundii*	291	643G→A		bla_{KPC-2}	JN048639.1 (17)
Argentina	*Enterobacter cloacae*	291	643G→A		bla_{KPC-2}	JN048640.1 (17)
Chile	*K. pneumoniae*	291	643G→A		bla_{KPC-2}	KR052100.1
Chile	*K. pneumoniae*	291	643G→A		bla_{KPC-24}	KR052099.1
China	*E. cloacae*	291	643G→A		bla_{KPC-3}	KU302800.1 (11)[c]
Singapore	*K. pneumoniae*	291	643G→A	11	bla_{KPC-2}	KC344543.1 (21)
Russia	*K. pneumoniae*	291	643G→A	11	bla_{KPC-2}	KP008371.1 (this study)[c]

[a]Deletions and SNPs of Δ_{TEM} fragments were identified in comparison with bla_{TEM-1} GenBank accession no. J01749 (22).
[b]Deletions in the intergenic region were identified in comparison with accession no. JN048639.1 (17).
[c]Full sequences of plasmids are available.

fragments, specific 291-bp deletions and single nucleotide polymorphisms (SNPs) (643G→A) were detected in Δ_{TEM}. An 11-bp deletion was detected in NCR_{211} from the Russian *K. pneumoniae* isolate and in an isolate from Singapore. Thus, the bla_{KPC-2}-harboring fragment from pKPCAPSS was most closely related to the NTE_{KPC}-IIa element according to the nomenclature, but it had an 11-bp deletion in NCR_{211} (4). This suggests a common origin for all elements with Δ_{TEM}.

The role of NTE_{KPC} elements in global dissemination of bla_{KPC} is largely unknown. The variety of NTE_{KPC} hosts shows the hitchhiking type of bla_{KPC} gene dissemination with these elements; in contrast, bla_{KPC} dissemination with Tn*4401* is associated with particular genetic lines, such as *K. pneumoniae* CC258. NTE_{KPC} elements were first described in 2006 in China (9). They were later reported in South America (17). Here, we present data regarding the emergence of a new mobile element localized on a mosaic plasmid and harboring an NTE_{KPC} fragment isolated from northwestern Russia, although it is likely to have been imported from Southeast Asia.

Accession number(s). The complete nucleotide sequence of pKPCAPSS was deposited in GenBank under accession no. KP008371.1.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01856-16.

TEXT S1, PDF file, 0.9 MB.

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