Characterization of pKP1433, a Novel KPC-2-Encoding Plasmid from Klebsiella pneumoniae Sequence Type 340

C. C. Papagiannitsis, a V. Miriagou, a P. Giakkoupi, a L. S. Tzouvelekis, a C. A. C Vatopoulos a–d
Department of Microbiology, National School of Public Health, Athens, a Laboratory of Bacteriology, Hellenic Pasteur Institute, Athens, b Department of Microbiology, Medical School, University of Athens, Athens, c Central Public Health Laboratory, Hellenic Centre of Disease Control and Prevention, Vari, d Greece

The nucleotide sequence of pKP1433 (55,417 bp), a bla_kpc-2-carrying plasmid from Klebsiella pneumoniae sequence type 340, was determined. pKP1433 displayed extensive sequence and structural similarities with the IncN plasmids possessing the KPC-2-encoding Tn4401b isoform. However, the replication, partitioning, and stability of pKP1433 were determined by sequences related to diverse non-IncN plasmids.

Most of the KPC-producing Klebsiella pneumoniae strains belong to sequence type 258 (ST258) or other STs of clonal complex 292 (1, 2). bla_kpc commonly occurs as part of the Tn4401a isoform carried by similar IncFIIK plasmids (3, 4). However, an increasing diversity of clonal lineages and plasmids is being observed among KPC producers (5). We have previously reported on the sporadic occurrence in Greek hospitals of K. pneumoniae ST340 isolates containing a plasmid-borne bla_kpc-2 gene associated with the Tn4401b isoform (5). We describe here the sequence of pKP1433, representing the KPC-2-encoding plasmids harbored by this distinct group of K. pneumoniae strains.

Plasmid pKP1433 could not be transferred by conjugation to Escherichia coli hosts (5) and was nontypeable by the PCR-based replicon typing (PBRT) method (6). Plasmid DNA was extracted from an E. coli DH5α transformant using the Qiagen large-construct kit (Qiagen, Hilden, Germany). Sequence analysis was performed by using the 454 genome sequencer FLX system on a standard DNA fragment library (Sistemas Genomicos, S.L., Valencia, Spain). The results were assembled into four contigs using 454 Newbler Assembler software (7). Gaps were filled by sequencing of PCR-produced fragments. The final contig assembly was carried out using Laser Gene software (DNAStar, Madison, WI). For sequence analysis and annotation, the BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST), insertion sequence (IS) finder (www-is.biotoul.fr/), and open reading frame (ORF) finder (www.bioinformatics.org/sms/) were utilized.

pKP1433 was 55,417 bp in size and included 59 coding sequences (48 complete and 11 truncated). The plasmid showed a complex structure, being mainly composed of an IncN-derived segment, as well as sequences of diverse origin, and was punctuated by mobile elements. A linear map of pKP1433 is shown in Figure 1.

A sequence of 13,836 bp (nucleotides [nt] 1 to 13836) shared common features with the backbone of the IncN prototype plasmids R46 (GenBank accession no. AY046276) and pKM101 (8), including kikA, korB, and two traA (TRAI and TRA II; 10.1 kb). Genes comprising TRA III were not found, explaining the inability of pKP1433 to transfer via conjugation. Also, pKP1433 carried sequences (nt 13837 to 26798 and 51415 to 55417) that have been found in the KPC-encoding IncN plasmids 9, 12, and pBK31551 from K. pneumoniae strains isolated in the United States (9, 10). Adjacent to TRA II and within the coding sequence of the mec gene, there was a truncated form of the KPC-2-encoding transposon Tn4401b (ΔTn4401b; 9,395 bp) that was 100% identical to that carried by plasmid 9 (9). ΔTn4401b consisted of ISKpn6, bla_kpc-2, ISKpn7, tnpA, and part of tnpR (ΔtnpR). The latter gene had been truncated by the insertion of a Tn1000/Tn25 hybrid transposon comprising a Tn1000-like tnpA and part of Tn25 tnpR. This segment was 97% identical to that found in plasmid 12, although the associated ars operon was not present in pKP1433. Genes encoding a restriction endonuclease, an EcoRII methylase, and an EcoRII endonuclease were located downstream from kikA, as in the aforementioned KPC-encoding plasmids and other multiresistant IncN plasmids, such as pNL194 (11), pKOX105 (12), and pKP96 (13). An intact group II intron (2,270 bp; nt 45033 to 47302) was identified upstream from the EcoRII endonuclease. It included an intron RNA that may promote self-splicing and intron mobility, as well as an ORF encoding a multifunctional protein with reverse transcriptase and RNA maturase activities required for site-specific DNA insertion (14–16). A similar region (90% identity) has also been described in plasmid 9, although it is disrupted by the insertion of a copy of Tn4401b (9).

pKP1433 lacked the replication region and stability operon that are characteristic of the IncN plasmids. However, in the 24,616-bp segment adjoining the boundaries of the presumably IncN-derived part (nt 26799 to 51414), sequences most probably implicated in essential plasmid functions were identified. A repB-like sequence of 693 bp exhibiting 79, 73, and 71% identity with the repB genes of pCTU3 from Cronobacter turicensis strain z3032 (GenBank accession no. FN543096) (17), p14-120 from Salmonella sp. strain 14 (GenBank accession no. JQ418538), and pPAT9B05 from Pantoea sp. strain At-9b (GenBank accession no. CP002438), respectively, was located at positions 39393 to 40085. None of the latter plasmids could be assigned to any of the known incompatibility groups, thus explaining the failure of the PBRT method to classify pKP1433. The putative repB product of
pKP1433 showed high amino acid sequence similarity (from 91 to 96%) with the replication initiation proteins of the aforementioned plasmids. The similarities with other replicases were significantly lower, the higher scores being observed with RepH11A (77%) (18) and RepFIB (65%) (19). Upstream from repB, a single-strand initiation sequence motif (ssIF) was present (nt 40421 to 40476). A resD-like gene, belonging to the family of the xerCD site-specific recombinases, was located at positions 37992 to 38768. Its putative product (resolvase) is probably involved in the site-specific recombinases, was located at positions 40419 to 40472. A plausible hypothesis is that, at a certain step of the evolution of pKP1433, the IncN formed with another plasmid a fusion multireplicon structure that, after resolution, evolved further through rearrangements facilitated by insertion sequences. Nevertheless, en block acquisition of an IncN-derived large segment containing Tn4401b by a plasmid carrying the repB gene cannot be excluded. Whichever is the case, the IS26 elements bordering the two distinct segments constituting pKP1433 most probably played an important role in shaping this novel plasmid. The findings of this study underscore the diversification potential of plasmids carrying important resistance determinants such as blaKPC.

**Nucleotide sequence accession number.** The complete nucleotide sequence of plasmid pKP1433 has been assigned GenBank accession no. JX397875.
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