

# Complete Nucleotide Sequence of the First KPC-2- and SHV-12-Encoding IncX Plasmid, pKpS90, from *Klebsiella pneumoniae*

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**We report the complete nucleotide sequence of the pKpS90 plasmid, carrying the *bla*<sub>KPC-2</sub> and *bla*<sub>SHV-12</sub> genes. This plasmid was isolated from a sequence type 258 (ST258) *Klebsiella pneumoniae* strain responsible for an outbreak in a French university hospital in 2009. pKpS90 is a 53,286-bp plasmid that belongs to the IncX incompatibility group. pKpS90 consists of a backbone from IncX plasmids, in which the KPC-2-encoding Tn4401 transposon and a *bla*<sub>SHV-12</sub>-encoding region have been inserted.**

During the past decade, carbapenem-resistant strains belonging to the *Enterobacteriaceae* have emerged and spread worldwide. Resistance to carbapenem arises by the acquisition of various  $\beta$ -lactamases belonging to the three Ambler classes A, B, and D. Since the initial report of a KPC  $\beta$ -lactamase from a strain of *Klebsiella pneumoniae* in 1996, KPC producers have been found in various regions. Current reports indicate that KPC-producing isolates of *K. pneumoniae* are widespread in China, Israel, Greece, South America, and the United States (1, 2).

Several KPC variants differing in one or two amino acids (KPC-1 to KPC-11) have been identified mainly in *K. pneumoniae* and to a lesser extent in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates. Among these variants, KPC-2 and KPC-3 are predominant (1, 3) (<http://www.lahey.org/studies/>). Molecular analysis of the genetic environment of KPC genes has shown that they were associated with the Tn4401 transposon, which is related to Tn3 and which has been detected among conjugative and nonconjugative plasmids (4, 5). Tn4401 is approximately 10 kb long, delimited by two 39-bp imperfect inverted repeat sequences, and harbors *bla*<sub>KPC</sub> gene, transposase and resolvase genes, and two insertion sequences, ISKpn6 and ISKpn7. To date, five isoforms (a, b, c, d, and e) of this transposon have been described, differing in deletions (3, 6–8). However, all Tn4401 transposons described to date have a largely conserved structures and sequences.

KPC-producing strains usually belong to sequence type 258 (ST258) (7, 8) of the international multilocus sequence typing scheme (9). Tn4401 has been shown to be inserted in different sites on conjugative and nonconjugative plasmids varying in size (12 to 80 kb) and incompatibility group (4, 5, 10, 11).

A KPC-2-producing *K. pneumoniae* ST258 strain was responsible for an outbreak at University Hospital Paul Brousse in July 2009 (12). The index case was a patient transferred from Athens, Greece. In addition to the *bla*<sub>KPC-2</sub> gene, the isolate produced the SHV-12 extended-spectrum beta-lactamase (ESBL) and the narrow-spectrum  $\beta$ -lactamases TEM-1 and OXA-9. A plasmid of approximately 50 kb, encoding *bla*<sub>KPC-2</sub> and *bla*<sub>SHV-12</sub>, was successfully transferred to *Escherichia coli*. Here we report the complete nucleotide sequence of this plasmid, pKpS90.

Plasmid pKpS90 was extracted from the *E. coli* electroporant

DH10B using the Qiagen Large construct kit (Qiagen, Courtaboeuf, France). Sequencing was performed using shotgun and 3-kb paired-end sequencing runs on a 454/Roche GS FLX analyzer (Roche, Basel, Switzerland). The resulting sequences were assembled to a unique scaffold, and predicted gaps were closed by PCR, followed by Sanger DNA sequencing using the BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) in an ABI Prism 310 DNA sequencer (Applied Biosystems). Gene prediction and annotation were performed using the CAAT-box tool (13).

pKpS90 is a circular plasmid of 53,286 bp containing 53 open reading frames and displaying a G+C content of 49% (Fig. 1). This plasmid encodes a replication initiator protein, RepB, which showed 85% amino acid identity with the RepB protein encoded by an IncX plasmid from *Morganella morganii* (GenBank accession no. YP004869670). *In silico* analysis showed that pKpS90 possessed a high degree of sequence identity and gene synteny across its entire scaffold with the recently published plasmid pIncX-SHV (GenBank accession no. JN247852) (14). Indeed, it showed 99% identity at the nucleotide level over most of its length, with the exception of a region of 12,500 bp. The latter region carried the *bla*<sub>KPC-2</sub> and *bla*<sub>SHV-12</sub> genes. *bla*<sub>KPC-2</sub> from pKpS90 was part of a Tn4401 platform identical to the 10-kb element found in plasmids carrying *bla*<sub>KPC-2</sub>, such as plasmids S15, p39SLMT, and pNYC, isolated from *K. pneumoniae* strains (GenBank accession no. FJ223606, HQ589350, and EU176011), or in plasmids carrying the *bla*<sub>KPC-3</sub> gene, such as pKpQIL, from a *K. pneumoniae* strain isolated in Israel (GenBank accession no. GU595196). The region carrying *bla*<sub>KPC-2</sub> is surrounded by genes encoding full-length transposases and a recombinase, as described previously (4, 5, 15). It corresponds to the variant of Tn4401 designated isoform a,

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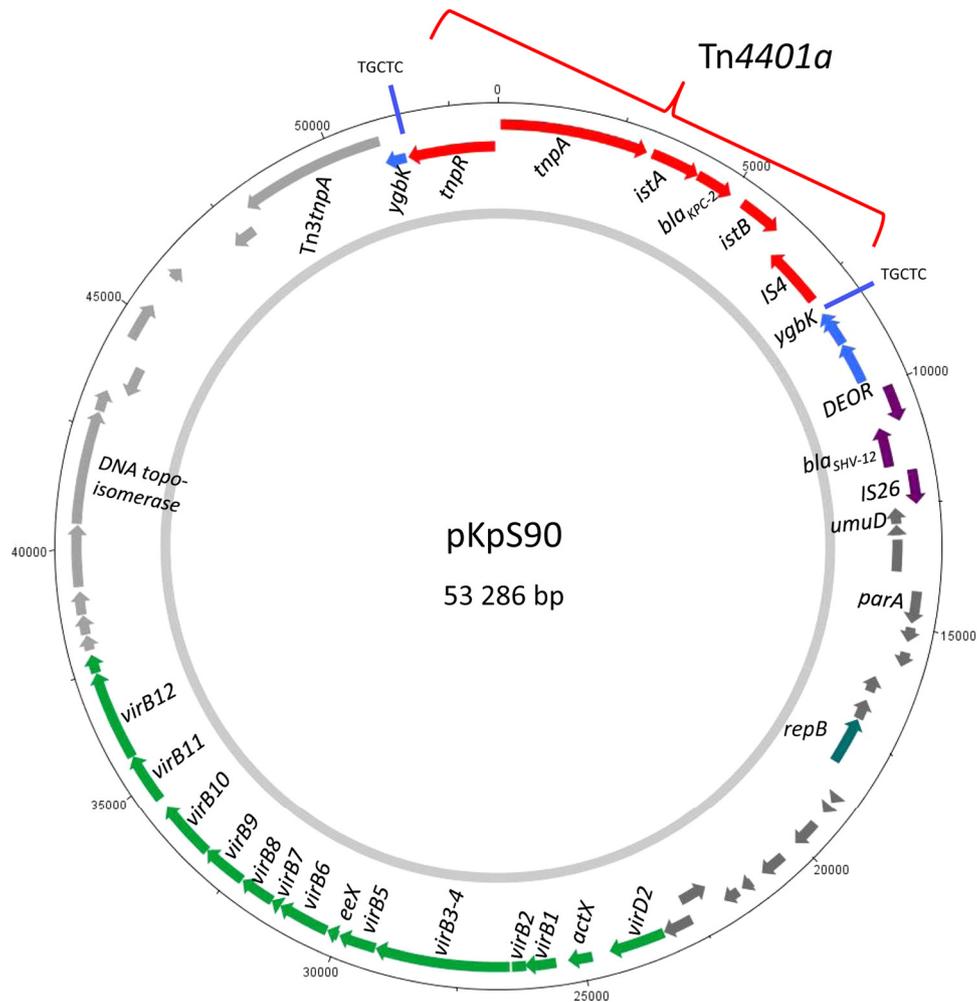


FIG 1 Circular map of plasmid pKpS90. Open reading frames are color coded as follows: red, Tn4401; purple, SHV-encoding transposon; green, *tra* locus; gray: other IncX backbone open reading frames (ORFs). Arrows show the direction of transcription.

which contains a 100-bp deletion between the *bla*<sub>KPC</sub> and *istB* genes.

The region surrounding *bla*<sub>SHV-12</sub> contains the following: (i) a gene encoding the transcriptional activator DEOR, which is usually associated with SHV-type ESBL, (ii) a part of the gene encoding a tRNA synthetase-like protein previously found to be originating with the *K. pneumoniae* chromosome, and (iii) a single copy of the IS26 insertion sequence, known to be critical for the mobilization of SHV-encoding genes (16). This region is identical to the one found on plasmid pRMH712 from *K. pneumoniae* strain 4003 (GenBank accession no. GU553923.1; positions 1542 to 6376).

The analysis of the region flanking the  $\beta$ -lactamase genes suggested that the insertion of Tn4401 occurred on a plasmid already carrying the SHV-12-encoding transposon. Indeed, the insertion occurred in the *ygbK* gene and generated 5-bp direct repeats (TGCTC) flanking the insertion sequences ISKpn6 and ISKpn7 from Tn4401. Moreover, a second strain of *K. pneumoniae* was isolated from urine of the same patient. This strain (Kp90-2) harbored the *bla*<sub>SHV-12</sub> gene but lacked *bla*<sub>KPC-2</sub>. We partially analyzed a 40-kb plasmid (pKp90-2) isolated from the Kp90-2 strain by performing PCR with specific primers matching various regions

of pKpS90, including those flanking *repB*, the *tra* region, and *ygbK*. Results confirmed the insertion of Tn4401 into pKp90-2 and suggested that pKpS90 and pKp90-2 had the same backbone.

The remaining part of pKpS90, which showed high homology with pIncX-SHV (GenBank accession no. JN247852), contained a partition system *parA* gene (similar to a partition gene from *Salmonella enterica* serotype Heidelberg strain SL486 [GenBank accession no. EDZ23062.1]) and a full *tra* region involved in plasmid transfer via a type IV secretion system (approximately 35 kb). Recently, comparative analysis of IncX plasmids demonstrated that these plasmids are much more prevalent among enteric bacteria than had previously been acknowledged. In addition, the study showed that IncX plasmids are subdivided into four different groups (IncX1, IncX2, IncX3, and IncX4), and a revised typing procedure was proposed (17). According to this typing procedure, pKpS90 was found to belong the IncX3 group, as reported for pIncX-SHV.

In conclusion, we report the first complete sequence of a plasmid encoding both the KPC-2 carbapenemase and the SHV-12 ESBL. Moreover, it is the first description of an IncX plasmid encoding KPC-2. Indeed, *bla*<sub>KPC</sub> genes have been previously found on plasmids from IncFII, IncN, IncL/M, or unknown

incompatibility groups (10, 11, 18). These findings expand the range of replicons onto which Tn4401 is able to transpose and contribute to explaining the worldwide dissemination of KPC  $\beta$ -lactamases.

**Nucleotide sequence accession number.** The complete DNA sequence of plasmid pKpS90 was assigned GenBank accession no. [JX461340](https://www.ncbi.nlm.nih.gov/nuclot/JX461340).

## ACKNOWLEDGMENTS

We have no conflict of interest to declare.

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