Characterization of pKP1780, a novel IncR plasmid from the emerging Klebsiella pneumoniae ST147, encoding the VIM-1 metallo-β-lactamase

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Objectives: To determine the complete nucleotide sequence of the VIM-1-encoding plasmid pKP1780 from Klebsiella pneumoniae representing a distinct group of IncR replicons.

Methods: The plasmid pKP1780 was from a K. pneumoniae clinical strain (KP-1780) isolated in Greece in 2009. Plasmid DNA was extracted from an Escherichia coli DH5α transformant and sequenced using the 454 Genome Sequencer GS FLX procedure on a standard fragment DNA library. Contig gaps were filled by sequencing of PCR-produced fragments. Annotation and comparative analysis were performed using software available on the Internet.

Results: Plasmid pKP1780 (49,770 bp) consisted of an IncR-related sequence (12,083 bp) including replication and stability systems, and a multidrug resistance (MDR) mosaic region (37,687 bp). blavim-1 along with the aacA7, dfrA1 and aadA1 cassettes comprised the variable region of an integron similar to In-e541 from pNL194. The mosaic structure also included the strA, strB, aphA1 and mphA resistance genes as well as intact (n = 10) or defective (n = 3) insertion sequences and fragments of various transposons.

Conclusions: The mosaic structure of pKP1780 exhibited high similarity with the acquired region of the IncN plasmid pNL194, indicating the acquisition of the VIM-1-encoding MDR region from pNL194 by an IncR-type plasmid.

Keywords: antibiotic resistance, carbapenemases, mosaic replicons

Introduction

VIM-1-producing Klebsiella pneumoniae clinical isolates were prevalent in Greece during the period 2001–07.1 In most isolates, blavim-1 was part of In-e5412 carried by self-transferable IncN plasmids,2,3 similar to the fully sequenced pNL194.4 Co-production of VIM-1 and KPC-2 was noticed for the first time in a clinical K. pneumoniae strain isolated in 2009.5 Subsequent studies indicated establishment of double carbapenemase producers in the flora of Greek hospitals. The majority of them have been classified in sequence type 147 (ST147).6 In these isolates, KPC-2 was encoded by IncFIIk plasmids, while blavim-1 was carried by non-transferable plasmids that could not be typed by the PCR-based replicon typing (‘PBRT’) method.7 We show here that the latter plasmids are similar, belonging to the novel IncR complex, and describe the sequence of pKP1780 representing this emerging VIM-1-encoding replicon.

Materials and methods

Thirty-six ST147 K. pneumoniae isolates (15 VIM-1-positive isolates and 21 VIM-1 plus KPC-2 producers as confirmed by PCR sequencing and isoelectric focusing) were included in the study. They had been recovered from hospitals throughout the country during 2009–10. Preliminary screening by an IncR-specific PCR assay8 using whole cell DNA showed that all 36 isolates were positive. Twenty of the double carbapenemase producers were also positive for both repFIIk and repFIB.9,10 The remaining isolate was positive only for repFIIk. To confirm the VIM–IncR association, plasmid DNA preparations extracted using a Qiagen Large-Construct Kit (Qiagen, Hilden, Germany) were used to transform Escherichia coli DH5α competent cells. We were able to obtain 16 transformants carrying single-plasmid species encoding VIM-1, but not KPC-2. They were also similar in size (~50 kb) and classified with the IncR group (Table S1, available as Supplementary data at JAC Online).

Plasmid pKP1780 from K. pneumoniae KP-1780 was selected for further characterization. An E. coli DH5α transformant was used as a source of
pKP1780 DNA. Sequence analysis was performed using the 454 Genome Sequencer GS FLX procedure on a standard fragment DNA library (Beckman Coulter Genomics SA, Grenoble, France). The results were assembled to eight contigs using the Mira assembler software. Sequence gaps on the plasmid were filled by sequencing of PCR-produced fragments. DNA Lasergene (DNASTAR, Madison, WI, USA), BLAST (www.ncbi.nlm.nih.gov/BLAST/), IS Finder (www.is.biotoul.fr/) and ORF Finder (www.bioinformatics.org/sms/) were used for data analysis.

The nucleotide sequence of plasmid pKP1780 has been assigned GenBank accession number JX424614.

Results and discussion

Plasmid pKP1780 was 49 770 bp in size with an average G + C content of 53.0%. Annotation of the finished sequence data revealed that pKP1780 contained 57 coding sequences (43 complete and 14 truncated), 47 of which encoded polypeptides similar to proteins with known functions. The coding sequences and their characteristics are presented in Table S2 (available as Supplementary data at JAC Online). A linear map of pKP1780 is shown in Figure 1.

pKP1780 included a contiguous segment of 12083 bp (nt 1–10300 and 47988–49770; GenBank accession number JX424614) sharing extensive similarity with sequences of the recently described replicons assigned to the novel IncR complex.8 A BLASTN search showed that the plasmidic scaffold of pKP1780 shared the highest similarity scores with the respective regions of pEFER (93% coverage; 99% identity) (GenBank accession number CU928144) and pK24511 (91% coverage; 99% identity), n recently described replicons assigned to the novel IncR complex.8

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Figure 1. Linear map of pKP1780 (GenBank accession number JX424614). Open reading frames (ORFs) are shown as rectangles (arrows within rectangles indicate the direction of transcription). Complete insertion sequences are presented as arrows, while truncated ones are shown as rectangles. The IncR-related sequences are shaded grey. Resistance genes are shown in pale blue and transposases are shown in red. The remaining genes in the non-IncR region are shown in white. Thick punctuated lines above the map denote the position of transposon-derived sequences; the respective inverted repeats (IRs) and target site duplications are also indicated. Thin lines below the map correspond to highly similar sequences carried by plasmids pEFER (IncR) and pNL194 (IncN).
extending from orf1780-3 to IS6100 of Tn1696 (nt 17 535–39 823 in pKPL780) (Figure 1). This segment included a remnant of Tn5393 including strA and strB (∆Tn5393-1), an IS903-B-like element truncated at the 5′ end (∆IS903.B-1†) similar to that described in pNL194, a second intact IS903-B-like element (IS903.B-2), an IS1222-like sequence, a truncated Tn2 (∆Tn2) and a fragment of Tn1721 (∆Tn1721-1).

Furthermore, the mosaic structure included an integron similar to In-e541 from pNL194 whose variable region comprised blaOXY-1, along with the aacA7, dfrA11 and aadA1 cassettes. As in pNL194, the 5′-CS and 3′-CS of the integron were disrupted by two copies of IS26. The 5′-CS-associated IS26 (IS26-4) bounded a ∆orf6-IS6100 sequence. Unlike pNL194, the 3′-CS-associated IS26 (IS26-3) comprised part of the Tn4352-like composite transposon (Figure 1). Yet an inverted Tn4352-like sequence has also been located in a different region of pNL194. The orientation of the Tn4352-like sequence suggested that IS26-mediated inversions may have repositioned the transposon in pKPL780. The second IS26 (IS26-2) of Tn4352-like was found adjacent to ∆Tn1721-1.

The remaining part of the mosaic structure consisted of two segments flanking the pNL194-like region (nt 10 301–17 534 and nt 39 824–4 7987). In the first of these, an IS1 was found at the boundary of the plasmidic backbone, 97 bp downstream of resA. Adjacent to IS1 was a segment of 674 bp similar to the replication region of IncN plasmids including the 3′ end of repA (∆repA) and 314 bp of the characteristic group 1 iterons. The IS1-∆repA-∆iterons structure was identified in pEFER at a similar position. Next to this sequence, the remaining part of IS903.B-1 (∆IS903.B-1†) and a second fragment of Tn5393 (∆Tn5393-2) comprising the IRR and part of trnA were found. Target site duplications of 5 bp (TTTAT) were identified at the boundaries of IRRs of Tn5393, indicating transposition of Tn5393 within IS903.B-1. The deleted part of Tn5393 was occupied by an IS26 (IS26-1), a truncated IScep1 element (∆IScep1) and an orf1780-3. In the second segment, a macrolide resistance operon [mph(A) region], including the mphR(A), mrx and mph(A) genes, was identified at the boundary of IS6100. A second 1718 bp fragment of Tn1721 (∆Tn1721-2) consisting of the IRL of the transposon and a truncated orf1 that lacked 60 bp of its 5′ end due to insertion of an IS26 (IS26-5) were found upstream of mph(A). A 916 bp fragment of an ISec15-like element (∆ISec15) was found at the boundary of the plasmid backbone, downstream of qacD. The remaining part of ∆ISec15 was probably deleted due to insertion of an ISec21-like element. The ISec21-∆ISec15 structure has also been observed in pEFER downstream of the IncR-associated qacD.

Several plasmids containing IncR characteristic sequences and carrying a variety of resistance genes such as qnrS1, blaoxy-2S, blaoxy-2 or blaOXY-1 have recently been described in enterobacterial isolates from different geographical regions. Identification of the IncR multiresistant pKPL780 in the present study further underscores the increasing clinical importance of this emerging plasmid family as well as the spreading potential of large MDR segments through reshuffling of enterobacterial plasmids.

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Transparency declarations
None to declare.

Supplementary data
Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

