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

C. C. Papagiannitsis1*, V. Miriagou2, P. Giakkoupi1, L. S. Tzouvelekis3 and A. C. Vatopoulos1,4

1Department of Microbiology, National School of Public Health, Athens, Greece; 2Laboratory of Bacteriology, Hellenic Pasteur Institute, Athens, Greece; 3Department of Microbiology, Medical School, University of Athens, Athens, Greece; 4Central Public Health Laboratory, Vari, Greece

*Corresponding author. Present address: Department of Microbiology, Faculty of Medicine and University Hospital in Plzen, Alej Svobody 80, Plzen 30460, Czech Republic. Tel: +420-603113354; Fax: +420-377103250; E-mail: c.papagiannitsis@gmail.com

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Objectives: To determine the complete nucleotide sequence of the VIM-1-encoding plasmid pKP1780 from Klebsiella pneumoniae ST147 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 (49770 bp) consisted of an IncR-related sequence (12083 bp) including replication and stability systems, and a multidrug resistance (MDR) mosaic region (37687 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,3,4 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 IncFIK 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 repFIK and repFIB.9,10 The remaining isolate was positive only for repFIK. 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
The nucleotide sequence of plasmid pKP1780 has been assigned GenBank accession number JX424614.

Results and discussion

Plasmid pKP1780 was 49770 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 exhibited the highest similarity scores with the respective regions of pEFER (93% coverage; 99% identity) (GenBank accession number CU928144) and pK24511 (91% coverage; 99% identity), containing nine resistance genes as well as intact (n = 3) insertion sequences and fragments of various transposons was identified. A BLASTN search showed that the mosaic structure of pKP1780 exhibited a high similarity score with the acquired region of the IncN multidrug resistance (MDR) plasmid pNL194 (60% coverage; 99% identity). Similarities between the two plasmids were localized at positions 2620–3486. The replication region also included a set of iterons (361 bp) controlling RepB expression and the plasmid copy number.12 A resD-like gene, encoding a resolvase of the site-specific recombinase family XerCD, was located at positions 761–1537. ResD probably contributes to multimter resolution, and thus could be important for plasmid maintenance.

A sequence, including parA and parB genes, was carried by pKP1780 as well as pEFER at a similar position (i.e. downstream of repB). The ParA and ParB proteins are involved in the partitioning and compatibility of the plasmid. Regions near the par genes possessed characteristic features such as the repeat sequences of the binding sites and the central integration host factor-binding motif of the Par family.13 Thus the parAB operon (nt 4348–6527) may be functional, contributing to pKP1780 maintenance. The virulence-associated genes vagD and vagC were identified upstream of repB, as in pEFER. The VagC and VagD proteins encoded by the vagCD operon (nt 48173–48816) are believed to be involved in the coordination of plasmid replication with host cell division.16

The plasmidic backbone also included a umuDC operon (nt 6609–8311) needed for maximal SOS mutagenesis.15 However, pKP1780 lacked a typical conjugative transfer system, explaining its inability to be transferred via conjugation. The relevant region was also absent in pEFER (GenBank accession number CU928144).

In the remaining 37687 bp sequence (nt 10301–47987), adjoining the boundaries of the IncR backbone, a mosaic structure containing nine resistance genes as well as intact (n = 10) or defective (n = 3) insertion sequences and fragments of various transposons was identified. A BLASTN search showed that the mosaic structure of pKP1780 exhibited a high similarity score with the acquired region of the IncN multidrug resistance (MDR) plasmid pNL194 (60% coverage; 99% identity). Similarities between the two plasmids were localized in a 22289 bp sequence,
extending from orf1780-3 to IS6100 of Tn1696 (nt 17535 – 39823 in pKP1780) (Figure 1). This segment included a remnant of Tn5393 including strA and strB (ΔTn5393-1), an IS903-B-like element truncated at the 5′ end (ΔIS903.B-1‘2) similar to that described in pNL194, a second intact IS903-B-like element (IS903-B-2), an IS1222-like sequence, a truncated Tn2 (ΔTn2) and a fragment of Tn1721 (ΔTn1721-1).

Furthermore, the mosaic structure included an integron similar to In-e541 from pNL194 whose variable region comprised blaoM-1, along with the aacA7, dfrA1 and aadA1 cassettes. 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 Δorf6-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 pKP1780. The second IS26 (IS26-2) of Tn4352-like was found adjacent to ΔTn1721-1.

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 – 47 987). In the first of these, an IS1 was found at the boundary of the plasmidic backbone. 97 bp downstream of retA. Adjacent to IS1 was a segment of 674 bp similar to the replication region of IncN plasmids including the 3′ end of repA (ΔrepA) and 314 bp of the characteristic group 1 iterons. The IS1-ΔrepA-Δiterons structure was identified in pEFER at a similar position. Next to this sequence, the remaining part of IS903-B-1 (ΔIS903.B-1‘1) and a second fragment of Tn5393 (ΔTn5393-2) comprising the IRR and part of tnpA were found. Target site duplications of 5 bp (TTTAT) were identified at the boundaries of IRs of Tn5393, indicating transposition of Tn5393 within IS903-B-1. The deleted part of Tn5393 was occupied by an IS26 (IS26-1), a truncated ISecp1 element (ΔISecp1) 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 (ΔTn1721-2) 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 mphA. A 916 bp fragment of an ISec15-like element (ΔISec15) was found at the boundary of the plasmid backbone, downstream of qacG. The remaining part of ΔISec15 was probably deleted due to insertion of an ISec12-like element. The ISec21-ΔISec15 structure has also been observed in pEFER downstream of the IncN-associated qacG.

Several plasmids containing IncR characteristic sequences and carrying a variety of resistance genes such as qnrS1, blaOmpT-52, blaoPC-2 or blaoJM-1 have recently been described in enterobacterial isolates from different geographical regions. Identification of the IncR multiresistant pKP1780 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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References


