Major role of pKpQIL-like plasmids in the early dissemination of KPC-type carbapenemases in the UK

M. Doumith[1*], J. Findlay[1], H. Hirani[1], K.L. Hopkins[1], D.M. Livermore[1,2], A. Dodgson[3] and N. Woodford[1]

[1]Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, National Infection Service, Public Health England, NW9 5EQ London, United Kingdom

[2]Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

[3]Public Health Laboratory, Manchester, United Kingdom

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Corresponding author:

Michel Doumith
AMRHAI Reference Unit, National Infection Service, Public Health England, NW9 5EQ London United Kingdom
Email: Michel.Doumith@phe.gov.uk
Tel: +44(0)20 8327 6834

Objectives: KPC-producing Enterobacteriaceae were first seen in the UK in 2003 and have been increasingly reported since 2010, largely owing to an ongoing outbreak in North-West England. We examined the role of clonal spread and plasmid transmission in their emergence.

Methods: Isolates comprised KPC-positive *Klebsiella pneumoniae* (n=33), *Escherichia coli* (n=7) and *Enterobacter* spp. (n=4) referred to the national reference laboratory between 2008 and 2010 from 17 UK centres, including three in North-West England. Isolates were typed by MLST. Plasmids were transferred by electroporation and characterised by PCR or sequencing. PCR screening assays were developed to distinguish plasmid pKpQIL variants.

Results: The *K. pneumoniae* isolates included 10 STs, of which three belonged to clonal group (CG) 258. CG258 (n=19) isolates were detected in 13 centres but accounted for only 7/19 (36.8%) of those from North-West England. Most KPC-producers (37/44, 84.1%), including 16/19 CG258 *K. pneumoniae* carried bla_{KPC} on $IncFII_{K2}$ plasmids. Sequencing of a subset of these plasmids (n=11) revealed similarities with published pKpQIL. One variant, pKpQIL-UK - identified in *K. pneumoniae* CG258 (n=5) and ST468 (n=1) isolates from distinct centres - had only a few nucleotide changes from classical pKpQIL, whereas pKpQIL-D1 (n=1) and pKpQIL-D2 (n=4), from isolates of various species in the North-West, harboured large variations reflecting replacement of the partitioning and replication functions and potentially thereby facilitating spread. PCR revealed that 36/37 (97.3%) $IncFII_{K2}$-type plasmids in KPC-positive isolates had pKpQIL markers.

Conclusions: pKpQIL-like plasmids played a major role in the early dissemination of KPC enzymes in the UK.

Introduction

KPC (*Klebsiella pneumoniae* carbapenemase) enzymes are geographically widespread and increasingly prevalent.[1, 2] The family includes 23 variants (KPC-2 to KPC-24), with KPC-2 and KPC-3 being globally predominant. They are mainly associated with *K. pneumoniae*, and in particular with the ST258 lineage, although production by other Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* spp. is increasingly reported.[3-8] *K. pneumoniae* with KPC enzymes have been endemic in the United States since the late 1990s with later, dramatic, spread in e.g. Israel, Greece and Italy.[9-13] The first KPC enzyme identified in the UK was KPC-4, found in 2003 in an *Enterobacter cloacae* complex isolate from Scotland.[14] Since then, KPC-positive organisms have been occasionally reported in various part of the country, with the first KPC-carrying ST258 *K. pneumoniae* isolate from Scotland in 2007.[15] Numbers of KPC-positive isolates rose substantially from 2010, largely due to an outbreak centred on the Greater Manchester area in North-West England.[16] This outbreak remains ongoing six years later. In contrast to most international KPC problems, which are largely associated with the ST258 *K. pneumoniae* clone, the North West England outbreak is unusual in being polyclonal: its KPC-positive *K. pneumoniae* isolates have diverse PFGE profiles and belong to multiple MLST types and the 'outbreak' also includes KPC-positive isolates belonging to other Enterobacteriaceae species, principally *Enterobacter* spp. and *Escherichia coli*.[1] *K. pneumoniae* clonal group (CG) 258 is dominant among KPC-positive isolates from elsewhere in the UK.[17]

The first fully-sequenced plasmid encoding a KPC enzyme was pKpQIL, a self-conjugative $IncFII_{K2}$ replicon type element from a *K. pneumoniae* ST258 isolate collected in Israel.[18] Later studies have suggested a major role for pKpQIL-like plasmids in the dissemination of KPC enzymes in Israel, Italy, Greece and the United States.[19-21]

In this study, 44 KPC-positive isolates referred to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in the early spread (2008-2010) of KPC enzymes in the UK were investigated, to examine the role of pKpQIL-like plasmids in their emergence.

Materials and Methods

Clinical isolates and transformants

Isolates (n=44) comprised KPC-positive *K. pneumoniae* (n=33), *E. coli* (n=7) and *Enterobacter* spp. (n=4) referred to PHE's AMRHAI Reference Unit between 2008 and 2010 from 17 centres in the UK, including three in the Greater Manchester area (Table 1). They comprised 19 of 27 geographically scattered isolates examined by the Unit between 2008 and 2010 and 25 representatives (out of the 214 referred) from the start of the North-West England outbreak, all collected in 2010. Plasmids were extracted by an alkaline lysis method and were transferred by electroporation into *E. coli* α-Select Strain (Bioline, London, UK) using a GenePulser electroporator (Bio-Rad, Hemel Hempstead, UK). Transformants were selected on Luria-Bertani agar supplemented with 1 mg/L ertapenem.

Antimicrobial susceptibility testing and molecular characterisation of KPC-producing isolates

MICs were determined by BSAC agar dilution[22] and with results interpreted according to EUCAST guidelines (http://www.eucast.org/clinical_breakpoints). Conventional MLST was performed as detailed in the *K. pneumoniae* MLST website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The bla_{KPC} gene and its direct environment were amplified as previously described[23] and sequenced using an ABI Genetic Analyser capillary platform 3130XL (Applied Biosystems, CA, USA). Plasmids were classified using PCR-based replicon typing (PBRT);[24] IncFII replicons were sub-grouped by replicon sequence typing (RST).[25]

Plasmid sequencing and bioinformatics analyses

The complete DNA sequences of 11 KPC-encoding plasmids from isolates randomly selected to proportionally represent the distribution of isolates from the outbreak (6/25, 24%) and other UK regions (5/19, 26%), were obtained using a 454-Genome Sequencer FLX (Roche, Branford, CT, USA) on libraries generated using plasmid DNA purified from *E. coli* α-select transformants according to the standard protocol for whole-genome shotgun sequencing, producing 250-bp reads. A draft assembly was produced *de novo* with Newbler 2.6 (Roche, Branford, CT, USA); plasmid sequences were further cleaned from residual genomic contigs by *in-silico* subtraction of the host *E. coli* published genome sequences (*E. coli* str. K12 substr. DH10B, Genbank CP000948).

Based on BLAST homologies, the published sequence of the archetypal pKpQIL plasmid (NC_014016) was used as a reference to identify nucleotide variations with Newbler 2.6. Single nucleotide polymorphisms (SNPs) and indels were inspected manually and those located in regions of homopolymers consisting of more than six units were excluded from the analysis. The MAUVE program (http://darlinglab.org/mauve/mauve.html) was used to re-order assembled contigs according to the pKpQIL sequence. Remaining gaps were closed by standard PCR and sequencing using manually-designed primers. Coding sequences were identified using Glimmer 2.13 (http://www.cs.jhu.edu/~genomics/Glimmer/) and gene functions were inferred based on homology searches with BLAST. Sequence homologies with published plasmids were checked by BLAST using the Blast Ring Image Generator (BRIG) software.[26] The reference plasmids included (i) pKpQIL (GenBank NC_014016), pGR-1504 (KF874496), pIT-01C03 (HG969995), pKp41 (CP012000), pKpQIL-10 (KJ146687), pKpQIL-531 (CP008833), pKpQIL-6e6 (CP014650), pUHKPC07 (CP011986), pUHKPC33 (CP011991), pG12-KPC2 (KU665642), pIT-01C22 (HG969997), pKpQIL-IT (JN233705), pGR-1870 (KF874498), pKPN207_p2(LT216438) and pIT-11C07 (HG969998) all of which were previously reported from CG258

K. pneumoniae isolates, (ii) pGR-3913 (KF874499), pKpQIL-9b8 (CP014765), pGR-1780 (KF874497) and pKpQIL-234 (KJ146689) which were variously reported from *K. pneumoniae* isolates belonging to ST35, ST37, ST147 and ST234, respectively, and (iii) pBK33689 (KU295133), pKpQIL-571 (CP014669) and pKpQIL-Ec (KJ146688), all of which were reported from *E. coli*.

The complete nucleotide sequences of plasmids pKpQIL-UK, pKpQIL-D1 and pKpQIL-D2 generated in this study were submitted to GenBank under the accession numbers KY798507, KY798505 and KY798506, respectively.

Detection of pKpQIL-like IncFII$_{K2}$ plasmids by PCR

PCR primers were designed to amplify size-distinguishable fragments from six markers on pKpQIL-like backbones; these markers were selected based on the comparison of available plasmid sequences (Table 2). They covered four distinct regions of the pKpQIL-backbone, comprising (i) both the *traI* and *traK* genes encoding the transfer-conjugation functions, (ii) the *bla*$_{KPC}$ carbapenemase gene, and (iii) a gene encoding a conserved hypothetical protein (Table 1). Primers targeting *parB* of pKpQIL and its homologue in pKpQIL-D2 were added to differentiate between these two plasmid variants. The 50S ribosomal protein gene *rplQ* was targeted as an internal PCR control from a chromosomal region conserved among Enterobacteriaceae (Table 2).

Amplification mixtures contained each of the primers described in Table 2 at a final concentration of 0.2 µM and were performed with the following cycling conditions: 95°C for 5 min, 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s and one final cycle at 72°C for 5 min.

Results

Characteristics of the KPC producers

Thirty-three of the 44 KPC producers including 24 *K. pneumoniae*, seven *E. coli* and two *Enterobacter* spp. harboured bla_{KPC-2} whereas nine *K. pneumoniae* had bla_{KPC-3} and two *Enterobacter* spp. carried bla_{KPC-4}. Almost all (41/44, 93.2%) of the KPC-encoding genes were embedded in Tn*4401* isoform 'a' transposons, predominantly on $IncFII_{K2}$ replicon-type plasmids (n=37), although some were carried on $IncFII_{K1}$ (n=1), $IncFII_{K5}$ (n=1) or non-typable (n=2) plasmids. Of the remainder, genes encoding KPC-3 (n=1) or KPC-4 (n=2) enzymes were located within Tn*4401* isoform 'b' transposons on non-typable plasmids. Among 27 isolates from North-West England, only four (14.8%) had their bla_{KPC} gene on non-$IncFII_{K2}$ plasmids; these comprised three *K. pneumoniae* and one *E. coli* carrying the gene on $IncFII_{K5}$ or non-typable plasmids.

The 33 *K. pneumoniae* isolates included 10 STs, with 19 isolates belonging to the clonal group (CG) 258 and comprising ST258 (n=15), ST11 (n=3) and ST512 (n=1) (Table 1). Non-CG258 STs (n=14) comprised ST321, with eight representatives mainly (6/8, 75%) from North-West England, as well as ST25, ST27, ST248, ST468, ST490 and ST491 each represented by a single isolate (Table 1). CG258 was widespread, being identified in 13 different centres across the UK. It dominated among *K. pneumoniae* isolates (12/14, 85.7%) recovered outside North-West England. By contrast *K. pneumoniae* isolates (n=19) from North-West England were diverse and belonged to nine different STs, with CG258 (7/19, 36.8%) and ST321 (6/19, 31.6%) the most represented (Table 1).

Antibiotic susceptibility testing showed that the majority of the KPC-producers were resistant to all β-lactams (88.6%) with the exception of five isolates recovered outside the North-West region showing susceptibility to meropenem, alone (n=4) or to both meropenem and imipenem (n=1). In contrast, isolates remained mostly susceptible to colistin (88.6%) and variably susceptible to amikacin

(70.5%), gentamicin (68.2%), ciprofloxacin (38.6%) and tigecycline (65.9%), with no marked regional differences in susceptibility frequencies. Colistin resistance was detected in only five *K. pneumoniae* isolates, including three from North-West England (Table 3).

The structure of KPC-encoding IncFII$_{K2}$ plasmids

The presence of IncFII$_{K2}$ plasmids among diverse STs of KPC-positive *K. pneumoniae*, as well as *E. coli* and *Enterobacter* spp. suggested that these were playing a major role in the early dissemination of KPC enzymes in the UK. Therefore, 11 IncFII$_{K2}$ plasmids originating from *K. pneumoniae* (n=9), *Escherichia coli* (n=1) and *Enterobacter* spp. (n=1) from the North-West outbreak and five other UK centres were fully sequenced (Table 1).

Plasmids, designated pKpQIL-UK, from *K. pneumoniae* ST486 isolate L33 (North-West England) and CG258 isolates T4, T6, T8, T13 and T27 (from centres outside North West England), were nearly identical to the archetypal IncFII$_{K2}$ KPC plasmid pKpQIL. At most, these plasmids were distinguished by 32 nucleotide variations (Figure 1A) and, of these, 22 were confined in a single non-coding region of 100 bp (positions 5270 to 5370 of pKpQIL) and were observed in plasmid T8 only. Based on the published annotation of pKpQIL, eight other variations found in the present plasmids were located in non-coding regions or were synonymous, having no effect on the amino acid sequences of the inferred gene products. A more significant variation was that all the plasmids from CG258 variants had a 1-bp deletion, creating a translational shift in a region predicted to encode a 130 aa hypothetical protein (positions 86406 to 86795 pKpQIL); the plasmids from CG258 isolates T4, T8 and L33 also had a substitution in the coding region of *bla*$_{KPC,}$ changing KPC-3 to a KPC-2 enzyme.

The remaining five IncFII$_{K2}$ plasmids, from isolates L27, L38, T19, LESC and LENT, all from the North-West England outbreak, harboured large DNA fragment substitutions. The remaining portions of these plasmids, representing 86.8% of the total length of plasmid T19 and 84.2% of the four other derived plasmids remained almost identical to pKpQIL with at most 25 nucleotide variations to distinguish them. Of these variations, eight, including mutations in the KPC-encoding gene, were identical to those observed in the pKpQIL-UK variant suggesting that plasmids pKpQIL-UK, -D1 and -D2 have evolved from the same ancestor.

The T19 plasmid, named pKpQIL-D1 (117,903 bp), which originated from an ST11 *K. pneumoniae* isolate, had the 11,260 bp fragment (positions 25,539 to 36,799 of pKpQIL) located between the insertion sequence IS*26* and the resolvase and carrying *bla*$_{TEM-1}$, truncated *bla*$_{OXA-9}$, mercuric resistance genes and the *vagCD* addiction system genes replaced by a 15,524 bp fragment encoding 14 ORFs comprising IS*66*, truncated IS*4321R*, Tn*3* transposase, resolvase and nine hypothetical proteins of which three showed low homologies to endonuclease, Na^+/H^+ antiporter and DNA binding proteins (Figure 1).

The plasmids from *K. pneumoniae* isolates L27 and L38, from *E. coli* LESC and *Enterobacter* LENT were similar to each other and were designated pKpQIL-D2 (111,742 bp). This plasmid shared the pKpQIL regions from 1 to 36,830 bp and from 56350 to 113,637 bp, but had the 19,520 bp fragment located between the addiction operon *vagCD* and the plasmid stability *stbA* gene replaced by a 17,625-bp fragment harbouring 19 genes (Figure 1). Although markedly different at the sequence level, some of the replacement genes encoded proteins homologous to those determined by the deleted pKpQIL fragment, such as the UmuC and UmuD SOS mutagenesis and repair proteins, the ParA and ParB plasmid-partitioning proteins and an origin of replication RepB (Figure 1B). Interestingly, the 'new' portion, encoding the plasmid-partitioning genes, has been described in other plasmid sequences deposited in the GenBank database and originating from various Enterobacteriaceae genera including

196 *Klebsiella* (KP008371), *Salmonella* (CP006054), *Escherichia* (KT845955), *Providencia* (JQ824049),
197 *Enterobacter* (CP011587) and *Proteus* (CP015347). This degree of dissemination suggests that ParA and
198 ParB, as encoded by pKpQIL-D2, may favour stable plasmid maintenance in diverse host species.

199 ***Comparison of pKpQIL-like*** IncFII$_{K2}$ ***plasmids***

200 Sequences of the present pKpQIL-UK, -D1 and -D2 plasmids were compared with similar (≥ 75% identity)
201 pKpQIL-like plasmids previously reported from *K. pneumoniae* (n=19) and *E. coli* (n=3) in Greece, Italy,
202 Norway and United States (Figure 1A).

203 The majority (15/19) of the published plasmids from *K. pneumoniae* were from CG258 isolates
204 with the four exceptions being from ST35, ST37, ST147 and ST234 organisms (Figure 1A). In 11/19
205 plasmids, described from CG258 (n=8), ST35 and ST147 isolates of various origins, differences were
206 limited to a few nucleotide variations from the archetypal pKpQIL, and were similar to those identified in
207 this study. The remaining eight plasmids, of which six were from CG258 isolates, had various genetic
208 rearrangements including insertions or deletions of insertion sequences resulting in the acquisition of
209 genes encoding mainly resistance genes, as well as inversions or substitutions in pKpQIL portions
210 carrying genes encoding the partitioning, transfer and conjugal activities, similar to those identified here
211 in pKpQIL-D2 (Figure 1A). The detection of plasmids almost identical to pKpQIL in non-CG258 isolates, (i)
212 as in the case of published plasmids pGR-3913 (ST35)[21] and pGR-1780 (ST147)[21] and (ii) as with pKpQIL-
213 UK (ST486) here, indicates that these plasmids have the potential to spread among distinct *K.*
214 *pneumoniae* lineages.

215 In contrast to the dominance of near-classical pKpQIL in *K. pneumoniae*, all the published
216 pKpQIL-like plasmids from *E. coli*, and those sequenced here, had major modifications in their
217 backbones. Like the pKpQIL-D1 present, both pKpQIL-Ec and pKPQIL-571 plasmids (originally described
218 from *E. coli* isolates in the United States)[20] had deletions in the pKpQIL region located between the IS*26*

element and genes encoding the restriction endonuclease units (Figure 1A). On the other hand, plasmid pBK33689 (KU295133), also described in United States, had the 5.5-kb region located upstream of the *repA* gene substituted by a fragment carrying among others a gene encoding an additional replication protein RepB (Figure 1A).

Distribution of pKpQIL-like plasmids in the UK

The screening assay for pKpQIL-like plasmids was validated on the 11 isolates harbouring the fully sequenced KPC plasmids. As expected, all isolates amplified the pKpQIL-markers *traI*, *traK*, *hyp* and bla_{KPC} whilst the banding pattern of *parB* varied according to whether pKpQIL or pKPQIL-D2 was present (Table 1). The assay was then applied to the remaining (n=26) KPC-positive clinical isolates that had been shown to carry $IncFII_{K2}$ plasmids by PCR-based replicon typing but where sequencing had not been performed. Nearly all (25/26, 96%) yielded the *traI*, *traK*, *hyp* and the bla_{KPC} fragments. Of these, 21 amplified also the partitioning *parB* fragments of either pKpQIL (n=15), or pKpQIL-D2 (n=6) while four had neither (Table 1). Only one isolate failed to amplify any pKpQIL-marker and yielded the fragments of only bla_{KPC} and the *rplQ* internal control, suggesting that the KPC gene is harboured on an $IncFII_{K2}$ plasmid distinct from pKpQIL. Overall, the assay inferred the presence of pKpQIL-like plasmids in seven *K. pneumoniae* STs and pKpQIL-D2 in three STs; only the most represented CG258 and ST321 lineages were associated with both variants.

This screening supports the view that pKpQIL plasmids were disseminating in the UK in the 2008-10 study period and suggests considerably plasticity in the region carrying their partitioning functions. The assay showed that only two of the five *E. coli* isolates carried the *parB* of pKpQIL-D2 and all four isolates failing to amplify any *parB* fragments belonged to *K. pneumoniae*. Although no clear association was found between species and amplification of the plasmid-partitioning gene types carried,

the presence of other types of modifications that could potentially affect the replication or segregation of these plasmids cannot be excluded, particularly in those plasmids profiled only by PCR and not by sequencing

Discussion

Our analysis revealed that IncFII$_{K2}$ pKpQIL-like elements played a major role in the early (2008-10) spread of KPC carbapenemases among diverse Enterobacteriaceae species in the UK. Plasmids related to archetypal IncFII$_{K2}$ pKpQIL were identified in 36/37 isolates carrying an IncFII$_{K2}$ replicon type and in 81.2% (36/44) of all KPC-bearing isolates included in this study.

Sequencing identified plasmids (designated pKpQIL-UK) that were nearly identical to each other and to published pKpQIL from *K. pneumoniae* isolates belonging to CG258 and ST468 from distinct UK centres, including in North-West England. Screening for pKpQIL markers inferred the presence of these classical forms of pKpQIL in *K. pneumoniae* isolates belonging to four other STs (e.g. ST25, 248, 321 and 491) among those identified in this study. Published plasmid sequences from non-CG258 isolates,[20, 21] and those reported in this study clearly suggest that highly conserved pKpQIL plasmids, although mainly associated with CG258, are able to spread among other lineages of *K. pneumoniae.*

Plasmids with large DNA fragment replacements in their pKpQIL-backbone were identified from the North-West England outbreak only. Critically, though, they were found not only in *K. pneumoniae* isolates, but also in *Enterobacter* spp. and *E. coli*. One variant, pKpQIL-D2, had lost a 19.5-kb DNA fragment of pKpQIL that carries genes encoding the plasmid partitioning and replication functions and had this replaced with a 17.6-kb fragment partly encoding similar functions. In contrast to the original *parAB* genes of pKpQIL, which seem to be confined to *K. pneumoniae*, the variants present in pKpQIL-D2 have been described previously in plasmids from various Enterobacteriaceae species and might favour the stable inheritance of this variant plasmid across diverse species.

A further variant plasmid, termed pKpQIL-D1, was identified in a single *K. pneumoniae* isolate, from North-West England and had the 11.2-kb DNA fragment of pKpQIL harbouring the antimicrobial and mercury resistance genes and the plasmid maintenance system VagCD substituted. Interestingly,

the fully-sequenced plasmids pKpQIL-Ec (KJ146688) and pKpQIL-571 (CP014669) from *E. coli* and pKpQIL-98b (CP014765) from *K. pneumoniae*, both recently described in the United States, harboured deletions located in the same region substituted in pKpQIL-D1. The system *vagCD* is thought to help plasmid maintenance by preventing the cell division until plasmid replication is complete.[27] The loss of the plasmid maintenance system in these pKpQIL-derivatives may have increased the chances of being acquired by hosts in which their replication may be less efficient.

Comparison of published pKpQIL-like sequences with those generated in this study showed that the pKpQIL-region between the IS*26* element and the genes encoding UmuCD are the most affected by modifications. The identification of isolates failing to amplify any of the *parB* genes sought in the screening assay for pKpQIL markers developed in this study further supports the inference of high plasticity in this region.

Overall, these results showed that CG258 *K. pneumoniae* with conserved pKpQIL-like plasmids played a major role in the early spread of KPC enzymes in multiple regions of the UK. Their distribution among further *K. pneumoniae* STs shows that these relatively conserved pKpQIL plasmids can spread among lineages of this species. We postulate that, in North-West England they evolved, mainly by modifications of portions encoding plasmid partitioning and replication activities. We suggest that this, in turn, facilitated their spread into various Enterobacteriaceae species, again notably in North-West England. The evolution of unusually transmissible pKpQIL-like plasmids in the early years after the first appearance of KPC enzymes in the UK could also explain the polyclonal nature of *K. pneumoniae* isolates from the ongoing North-West England outbreak, as compared with the international experience, where the epidemiology of KPC *K. pneumoniae* is dominated by CG258 *K. pneumoniae* variants.

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Disclaimer

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382 Table 1. Molecular characterisation of the KPC-positive Enterobacteriaceae.

Isolate	Species	Year	ST	Centre	KPC variant	Tn*4401* Isoform	Replicon type	pKpQIL PCR							
								parB-O	KPC	rplQ	traK	traI	parB-D2	hyp	interpretation
T1	*K. pneumoniae*	2008	512 (CG258)	A	3	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T2	*K. pneumoniae*	2008	258 (CG258)	B	2	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T3	*K. pneumoniae*	2008	258 (CG258)	C	3	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T4	***K. pneumoniae***	**2008**	**258 (CG258)**	**D**	**2**	**a**	**IncFII$_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T5	*K. pneumoniae*	2009	258 (CG258)	E	3	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T6	***K. pneumoniae***	**2009**	**258 (CG258)**	**F**	**3**	**a**	**IncFII$_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
L16	*K. pneumoniae*	2009	258 (CG258)	NW-C2	2	a	IncFII$_{K2}$		+	+	+	+		+	pKpQIL-like*
T8	***K. pneumoniae***	**2009**	**258 (CG258)**	**G**	**2**	**a**	**IncFII$_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T9	*Enterobacter spp.*	2009	ND	H	4	b	ND	NT	NT	NT	NT	NT	NT	NT	unknown
T10	*K. pneumoniae*	2009	258 (CG258)	I	3	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T11	*K. pneumoniae*	2009	258 (CG258)	NW-C3	2	a	IncFII$_{K5}$	NT	NT	NT	NT	NT	NT	NT	unknown
T12	*K. pneumoniae*	2009	258 (CG258)	A	3	a	incFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
T13	***K. pneumoniae***	**2009**	**11 (CG258)**	**j**	**3**	**a**	**IncFII$_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T14	*K. pneumoniae*	2010	258 (CG258)	NW-C1	3	b	ND	NT	NT	NT	NT	NT	NT	NT	unknown
T15	*K. pneumoniae*	2010	321	E	2	a	incFII$_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
T16	*K. pneumoniae*	2010	321	NW-C1	2	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like

T17	*K. pneumoniae*	2010	248	NW-C1	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like
T18	*K. pneumoniae*	2010	321	K	2	a	$IncFII_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
T19	***K. pneumoniae***	**2010**	**11 (CG258)**	**NW-C1**	**2**	**a**	**$IncFII_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T20	*E. coli*	2010	ND	NW-C1	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like
T21	*E. coli*	2010	ND	NW-C1	2	a	ND	NT	NT	NT	NT	NT	NT	NT	Unknown
T22	*Enterobacter spp.*	2010	ND	NW-C1	2	a	$IncFII_{K2}$		+	+					Unknown
T23	*Enterobacter spp.*	2010	ND	L	4	b	ND	NT	NT	NT	NT	NT	NT	NT	Unknown
T24	*K. pneumoniae*	2010	321	NW-C3	2	a	$IncFII_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
T25	*K. pneumoniae*	2010	27	NW-C3	2	a	ND	NT	NT	NT	NT	NT	NT	NT	Unknown
T26	*K. pneumoniae*	2010	321	NW-C3	2	a	$IncFII_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
T27	***K. pneumoniae***	**2010**	**258 (CG258)**	**M**	**2**	**a**	**$IncFII_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T28	*K. pneumoniae*	2010	258 (CG258)	C	3	a	$IncFII_{K1}$	NT	NT	NT	NT	NT	NT	NT	Unknown
T29	*K. pneumoniae*	2010	321	NW-C1	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like
T30	*K. pneumoniae*	2010	321	NW-C1	2	a	$IncFII_{K2}$		+	+	+	+		+	pKpQIL-like*
T31	*E. coli*	2010	ND	NW-C1	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like
T32	*E. coli*	2010	ND	NW-C1	2	a	$IncFII_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
T33	*E. coli*	2010	ND	NW-C1	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like
L19	*K. pneumoniae*	2010	258 (CG258)	NW-C1	2	a	$IncFII_{K2}$		+	+	+	+		+	pKpQIL-like*
L22	*K. pneumoniae*	2010	11 (CG258)	NW-C1	2	a	$IncFII_{K2}$		+	+	+	+	+	+	pKpQIL-like (D2)
L23	*K. pneumoniae*	2010	25	NW-C2	2	a	$IncFII_{K2}$	+	+	+	+	+		+	pKpQIL-like

L27	***K. pneumoniae***	**2010**	**321**	**NW-C1**	**2**	**a**	**IncFII$_{K2}$**		**+**	**+**	**+**	**+**	**+**	**+**	**pKpQIL-like (D2)**
L33	***K. pneumoniae***	**2010**	**468**	**NW-C2**	**2**	**a**	**IncFII$_{K2}$**	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
L37	*K. pneumoniae*	2010	491	NW-C1	2	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like
L38	***K. pneumoniae***	**2010**	**490**	**NW-C1**	**2**	**a**	**IncFII$_{K2}$**		**+**	**+**	**+**	**+**	**+**	**+**	**pKpQIL-like (D2)**
L39	*K. pneumoniae*	2010	258 (CG258)	NW-C1	2	a	IncFII$_{K2}$		+	+	+	+		+	pKpQIL-like*
LENT	***Enterobacter spp.***	**2010**	**ND**	**NW-C1**	**2**	**a**	**IncFII$_{K2}$**		**+**	**+**	**+**	**+**	**+**	**+**	**pKpQIL-like (D2)**
LESC	***E. coli***	**2010**	**ND**	**NW-C1**	**2**	**a**	**IncFII$_{K2}$**		**+**	**+**	**+**	**+**	**+**	**+**	**pKpQIL-like (D2)**
I2	*E. coli*	2010	ND	N	2	a	IncFII$_{K2}$	+	+	+	+	+		+	pKpQIL-like

Isolates with sequenced KPC-encoding plasmids are shown in bold. (*) indicates plasmids failing to amplify any *parB* fragments; (ND) not determined; (NT) not tested. Centres is North-West England have codes starting NW-C; the other centres A-N were elsewhere in the UK

386 Table 2. Primers used to seek pKpQIL-like plasmids.

Target	Primer	Sequence	Size (bp)
hyp	hyp-for	GGTCAGAAAATCACGTCTGAA	412
	hyp-rev	CTCACCGTGAATGTCATAGC	
parB-D2	parBD2-for	GTAAGACCTTCGTAAACCAGGA	315
	parBD2-rev	AAGAGCGATCAATCTCAGGC	
traI	traI-for	TCGTTGCTCTCGTGTTTTTC	247
	traI-Rev	GGTGAAACCAGAATGACCAC	
traK	traK-for	CAGGCAAATATTGCCGTGAG	203
	traK-rev	GCACGAATGGAGAAGTTCAG	
rplQ	IC-for	ATGCGCCATCGTAAGAGTGGT	170
	IC-rev	GTCTTGGCAAGAGTAATCAGCGG	
*bla*KPC	KPC-for	GCTTGCTGGACACACCCAT	127
	KPC-rev	ATCACTGTATTGCACGGCG	
parB-O	parB-for	ACCTATGAATTTGCCCGTCT	91
	parB-rev	TTTCGAAGGACTGCATGTTG	

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388 Table 3. MIC distributions for KPC producers

Antibiotic	Species	Region	Number of isolates with MIC (mg/L)							% S	
			≤ 0.5	1	2	4	8	16	≥ 32		
Ertapenem	*K. pneumoniae*	North-West England							19	0	0
		Other centres							14	0	
	Other spp.	North-West England			1	1	2	1	3	0	
		Other centres				2[b]			1	0	
Imipenem	*K. pneumoniae*	North-West England					2	8	9	0	2.3
		Other centres					1	2	11	0	
	Other spp.	North-West England				2	4		2	0	
		Other centres		1		1		1		33.3	
Meropenem	*K. pneumoniae*	North-West England					3	7	9	0	11.4
		Other centres					1		13	0	
	Other spp.	North-West England		1	2	2		1	2	37.5	
		Other centres	1	1			1			66.7	
Cefotaxime	*K. pneumoniae*	North-West England					5	6	8	0	0
		Other centres						1	13	0	
	Other spp.	North-West England			1	1		2	4	0	
		Other centres							3	0	
Ceftazidime	*K. pneumoniae*	North-West England				1	2	6	10	0	0

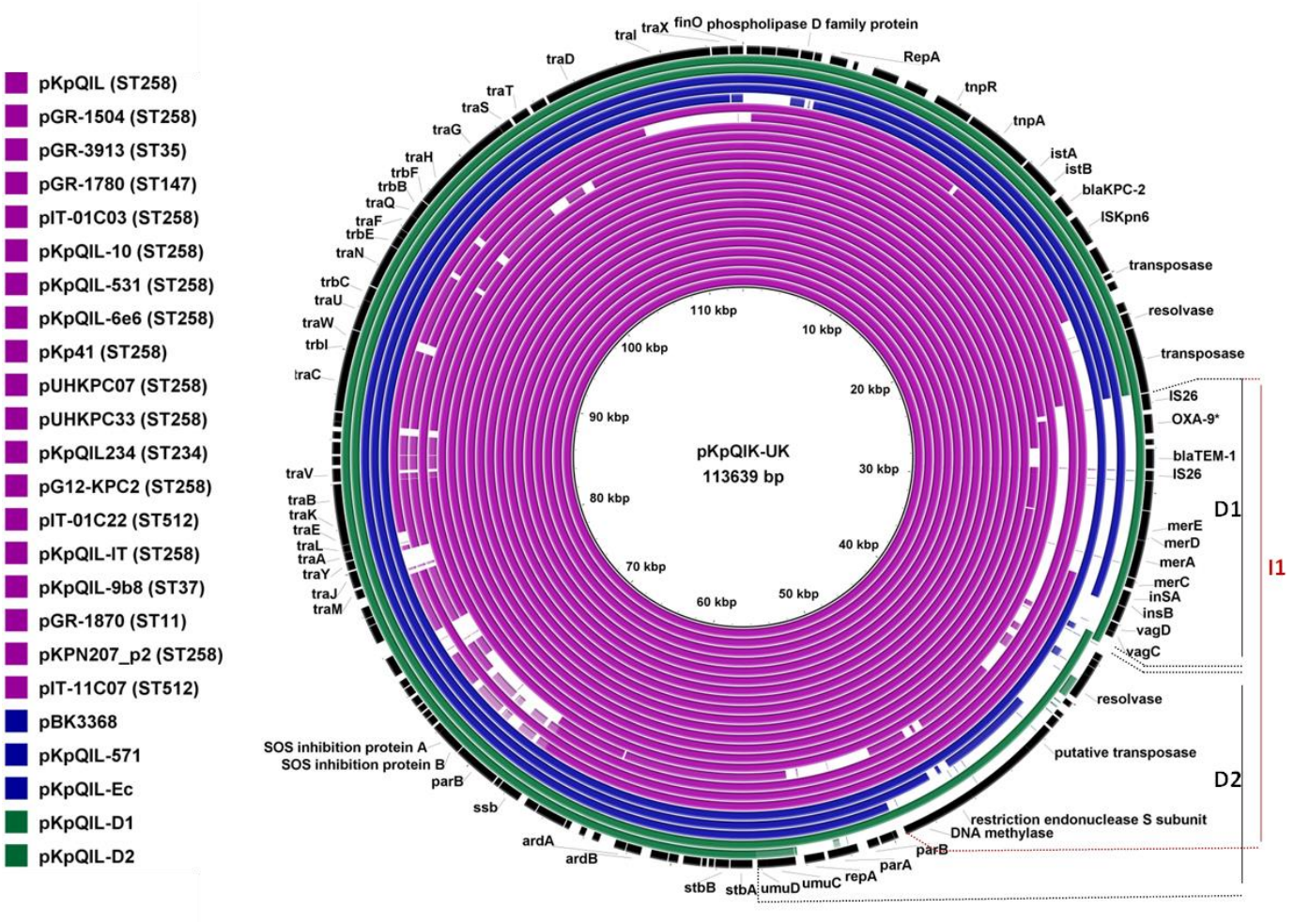
		Other centres						3	11	0		
	Other spp.	North-West England			2	1		2	3	0		
		Other centres					1		2	0		
Amikacin	*K. pneumoniae*	North-West England		5	6	1	2		5	73.7	70.5	
		Other centres	1	1	1	1	3	1	6	50		
	Other spp.	North-West England		1	3	2	2			100		
		Other centres		2					1	66.7		
Gentamicin	*K. pneumoniae*	North-West England	8	4	2		2		3	73.7	68.2	
		Other centres	2	5	2	2	2	1		64.3		
	Other spp.	North-West England	2	3		2			1	62.5		
		Other centres	2						1	66.7		
Ciprofloxacin	*K. pneumoniae*	North-West England	8	1	1	1	1	7[a]		42.1	38.6	
		Other centres	2					12[a]		14.3		
	Other spp.	North-West England	7					1[a]		87.5		
		Other centres						3[a]		0		
Colistin	*K. pneumoniae*	North-West England	12	4				1	2	84.2	88.6	
		Other centres	11	1			1		1	85.7		
	Other spp.	North-West England	8							100		
		Other centres	3							100		
Tigecycline	*K. pneumoniae*	North-West England	6	8	5					73.7	65.9	

	Other centres	1	5	7	1			42.9
Other spp.	North-West England	5	3					100
	Other centres		1		1	1		33.3

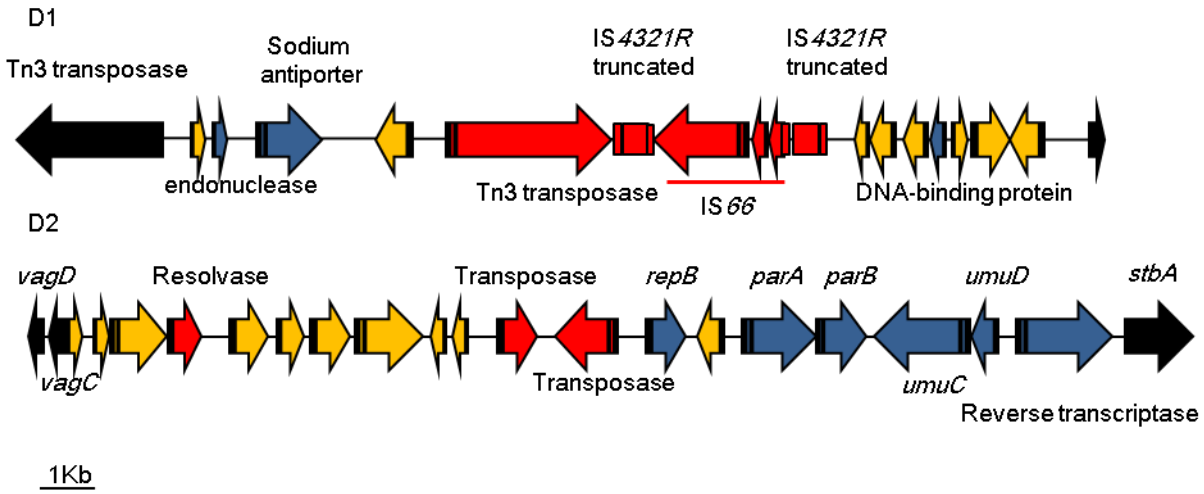
S, susceptible; cell colours showed number of isolates susceptible (white), intermediate (light-grey) and resistant (dark-grey) to each tested antibiotics according to EUCAST breakpoints. ([a]), showed numbers with MIC greater or equal to the indicated value, which represented the maximum concentration tested for the corresponding antibiotic, ([b]) indicated the ertapenem MIC of the *Enterobacter cloacae* isolate showing sensitivity to imipenem and meropenem.

Figure 1. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC

A. Sequence homologies amongst published pKpQIL-like plasmids.



Gene contents of the substituted DNA fragments of pKpQIL-D1 and pKpQIL-D2.

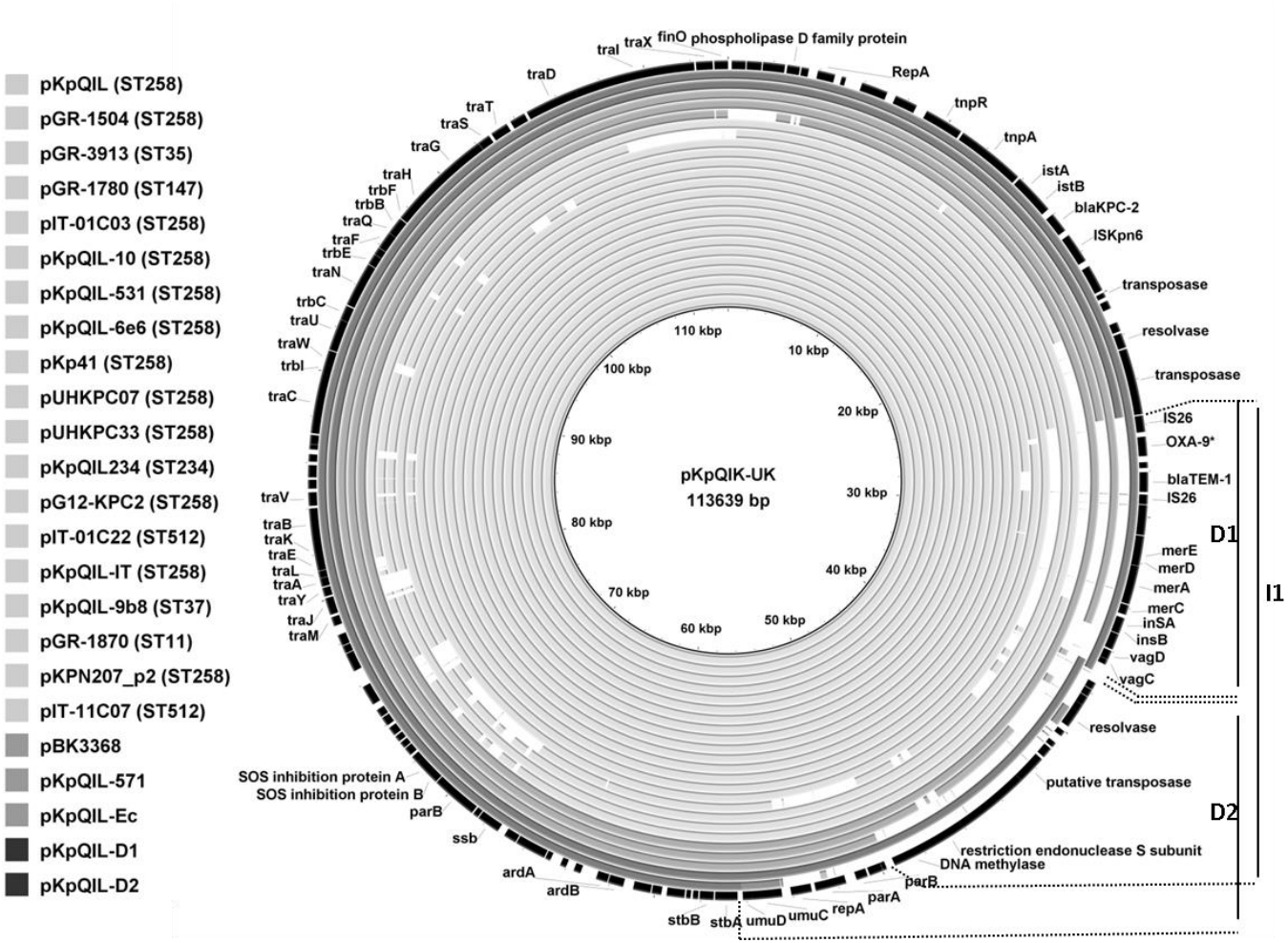


(A) Coloured rings showed published plasmids from *K. pneumoniae* (purple), *E. coli* (blue) and those generated in this study (green). D1 and D2 indicated the fragments of pKpQIL plasmid that were substituted in pKpQIL-D1 and pKpQIL-D2, respectively, while the pKpQIL fragment indicated I1 is inversely oriented in plasmid pKpQIL-234.

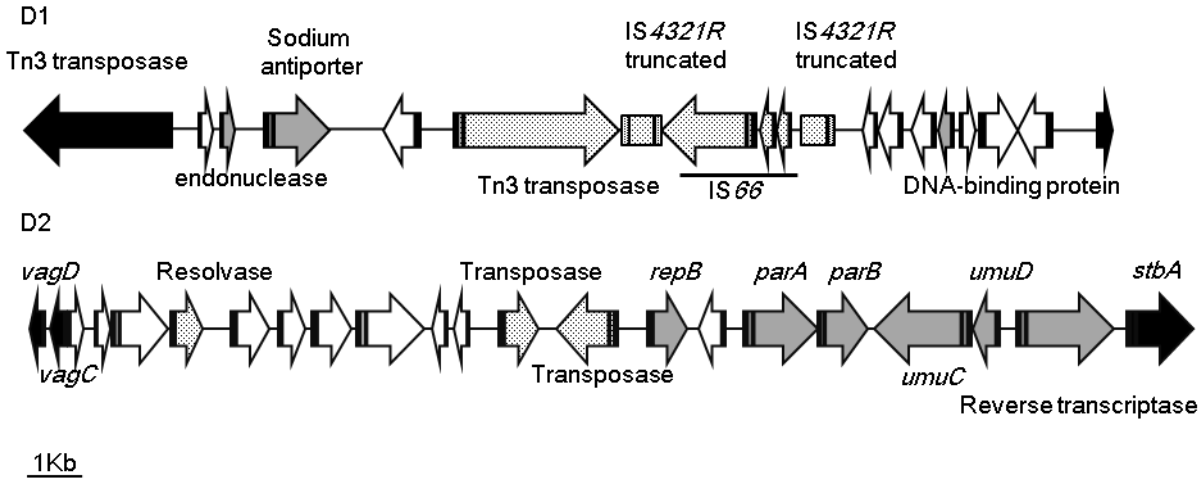
(B) Colours in the substituted DNA fragments showed genes encoding known functions (blue), hypothetical proteins (yellow) and mobile elements (red). Irrespective of their encoding functions, genes of pKpQIL present at the flanking regions of the two substitutions are coloured in black.

Figure 1. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC

A. Sequence homologies amongst published pKpQIL-like plasmids.



B. Gene contents of the substituted DNA fragments of pKpQIL-D1 and pKpQIL-D2.



(A) Coloured rings showed published plasmids from *K. pneumoniae* (light grey), *E. coli* (medium-dark grey) and those generated in this study (dark grey). D1 and D2 indicated the fragments of pKpQIL plasmid that were substituted in pKpQIL-D1 and pKpQIL-D2, respectively, while the pKpQIL fragment indicated I1 is inversely oriented in plasmid pKpQIL-234.

(B) Colours in the substituted DNA fragments showed genes encoding known functions (grey), hypothetical proteins (white) and mobile elements (black dots). Irrespective of their encoding functions, genes of pKpQIL present at the flanking regions of the two substitutions were coloured in black.