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

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10 **Running title:** KPC carbapenemase in the UK

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

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

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

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

41

42 **Introduction**

43 KPC (*Klebsiella pneumoniae* carbapenemase) enzymes are geographically widespread and increasingly
44 prevalent.[1, 2] The family includes 23 variants (KPC-2 to KPC-24), with KPC-2 and KPC-3 being globally
45 predominant. They are mainly associated with *K. pneumoniae*, and in particular with the ST258 lineage,
46 although production by other Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* spp. is increasingly
47 reported.[3-8] *K. pneumoniae* with KPC enzymes have been endemic in the United States since the late
48 1990s with later, dramatic, spread in e.g. Israel, Greece and Italy.[9-13] The first KPC enzyme identified in
49 the UK was KPC-4, found in 2003 in an *Enterobacter cloacae* complex isolate from Scotland.[14] Since then,
50 KPC-positive organisms have been occasionally reported in various part of the country, with the first
51 KPC-carrying ST258 *K. pneumoniae* isolate from Scotland in 2007.[15] Numbers of KPC-positive isolates
52 rose substantially from 2010, largely due to an outbreak centred on the Greater Manchester area in
53 North-West England.[16] This outbreak remains ongoing six years later. In contrast to most international
54 KPC problems, which are largely associated with the ST258 *K. pneumoniae* clone, the North West
55 England outbreak is unusual in being polyclonal: its KPC-positive *K. pneumoniae* isolates have diverse
56 PFGE profiles and belong to multiple MLST types and the 'outbreak' also includes KPC-positive isolates
57 belonging to other Enterobacteriaceae species, principally *Enterobacter* spp. and *Escherichia coli*.[1] *K.*
58 *pneumoniae* clonal group (CG) 258 is dominant among KPC-positive isolates from elsewhere in the UK.[17]

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

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

66

67 **Materials and Methods**

68 ***Clinical isolates and transformants***

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

77

78 ***Antimicrobial susceptibility testing and molecular characterisation of KPC-producing isolates***

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

85 ***Plasmid sequencing and bioinformatics analyses***

86 The complete DNA sequences of 11 KPC-encoding plasmids from isolates randomly selected to
87 proportionally represent the distribution of isolates from the outbreak (6/25, 24%) and other UK regions
88 (5/19, 26%), were obtained using a 454-Genome Sequencer FLX (Roche, Branford, CT, USA) on libraries
89 generated using plasmid DNA purified from *E. coli* α-select transformants according to the standard
90 protocol for whole-genome shotgun sequencing, producing 250-bp reads. A draft assembly was
91 produced *de novo* with Newbler 2.6 (Roche, Branford, CT, USA); plasmid sequences were further
92 cleaned from residual genomic contigs by *in-silico* subtraction of the host *E. coli* published genome
93 sequences (*E. coli* str. K12 substr. DH10B, Genbank CP000948).

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

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

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

115

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

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

125 Amplification mixtures contained each of the primers described in Table 2 at a final
126 concentration of 0.2 µM and were performed with the following cycling conditions: 95°C for 5 min, 30
127 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.

128

129 **Results**

130 ***Characteristics of the KPC producers***

131 Thirty-three of the 44 KPC producers including 24 *K. pneumoniae*, seven *E. coli* and two *Enterobacter*
132 spp. harboured bla_{KPC-2} whereas nine *K. pneumoniae* had bla_{KPC-3} and two *Enterobacter* spp. carried
133 bla_{KPC-4}. Almost all (41/44, 93.2%) of the KPC-encoding genes were embedded in Tn*4401* isoform 'a'
134 transposons, predominantly on $IncFII_{K2}$ replicon-type plasmids (n=37), although some were carried on
135 $IncFII_{K1}$ (n=1), $IncFII_{K5}$ (n=1) or non-typable (n=2) plasmids. Of the remainder, genes encoding KPC-3 (n=1)
136 or KPC-4 (n=2) enzymes were located within Tn*4401* isoform 'b' transposons on non-typable plasmids.
137 Among 27 isolates from North-West England, only four (14.8%) had their bla_{KPC} gene on non-$IncFII_{K2}$
138 plasmids; these comprised three *K. pneumoniae* and one *E. coli* carrying the gene on $IncFII_{K5}$ or non-
139 typable plasmids.

140 The 33 *K. pneumoniae* isolates included 10 STs, with 19 isolates belonging to the clonal group
141 (CG) 258 and comprising ST258 (n=15), ST11 (n=3) and ST512 (n=1) (Table 1). Non-CG258 STs (n=14)
142 comprised ST321, with eight representatives mainly (6/8, 75%) from North-West England, as well as
143 ST25, ST27, ST248, ST468, ST490 and ST491 each represented by a single isolate (Table 1). CG258 was
144 widespread, being identified in 13 different centres across the UK. It dominated among *K. pneumoniae*
145 isolates (12/14, 85.7%) recovered outside North-West England. By contrast *K. pneumoniae* isolates
146 (n=19) from North-West England were diverse and belonged to nine different STs, with CG258 (7/19,
147 36.8%) and ST321 (6/19, 31.6%) the most represented (Table 1).

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

152 (70.5%), gentamicin (68.2%), ciprofloxacin (38.6%) and tigecycline (65.9%), with no marked regional
153 differences in susceptibility frequencies. Colistin resistance was detected in only five *K. pneumoniae*
154 isolates, including three from North-West England (Table 3).

155

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

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

162 Plasmids, designated pKpQIL-UK, from *K. pneumoniae* ST486 isolate L33 (North-West England)
163 and CG258 isolates T4, T6, T8, T13 and T27 (from centres outside North West England), were nearly
164 identical to the archetypal IncFII$_{K2}$ KPC plasmid pKpQIL. At most, these plasmids were distinguished by
165 32 nucleotide variations (Figure 1A) and, of these, 22 were confined in a single non-coding region of 100
166 bp (positions 5270 to 5370 of pKpQIL) and were observed in plasmid T8 only. Based on the published
167 annotation of pKpQIL, eight other variations found in the present plasmids were located in non-coding
168 regions or were synonymous, having no effect on the amino acid sequences of the inferred gene
169 products. A more significant variation was that all the plasmids from CG258 variants had a 1-bp deletion,
170 creating a translational shift in a region predicted to encode a 130 aa hypothetical protein (positions
171 86406 to 86795 pKpQIL); the plasmids from CG258 isolates T4, T8 and L33 also had a substitution in the
172 coding region of *bla*$_{KPC,}$ changing KPC-3 to a KPC-2 enzyme.

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

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

186 The plasmids from *K. pneumoniae* isolates L27 and L38, from *E. coli* LESC and *Enterobacter* LENT
187 were similar to each other and were designated pKpQIL-D2 (111,742 bp). This plasmid shared the
188 pKpQIL regions from 1 to 36,830 bp and from 56350 to 113,637 bp, but had the 19,520 bp fragment
189 located between the addiction operon *vagCD* and the plasmid stability *stbA* gene replaced by a 17,625-
190 bp fragment harbouring 19 genes (Figure 1). Although markedly different at the sequence level, some of
191 the replacement genes encoded proteins homologous to those determined by the deleted pKpQIL
192 fragment, such as the UmuC and UmuD SOS mutagenesis and repair proteins, the ParA and ParB
193 plasmid-partitioning proteins and an origin of replication RepB (Figure 1B). Interestingly, the 'new'
194 portion, encoding the plasmid-partitioning genes, has been described in other plasmid sequences
195 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*

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

223

224 ***Distribution of pKpQIL-like plasmids in the UK***

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

237 This screening supports the view that pKpQIL plasmids were disseminating in the UK in the
238 2008-10 study period and suggests considerably plasticity in the region carrying their partitioning
239 functions. The assay showed that only two of the five *E. coli* isolates carried the *parB* of pKpQIL-D2 and
240 all four isolates failing to amplify any *parB* fragments belonged to *K. pneumoniae*. Although no clear
241 association was found between species and amplification of the plasmid-partitioning gene types carried,

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

245

246 **Discussion**

247 Our analysis revealed that $IncFII_{K2}$ pKpQIL-like elements played a major role in the early (2008-10) spread
248 of KPC carbapenemases among diverse Enterobacteriaceae species in the UK. Plasmids related to
249 archetypal $IncFII_{K2}$ pKpQIL were identified in 36/37 isolates carrying an $IncFII_{K2}$ replicon type and in
250 81.2% (36/44) of all KPC-bearing isolates included in this study.

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

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

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

269 the fully-sequenced plasmids pKpQIL-Ec (KJ146688) and pKpQIL-571 (CP014669) from *E. coli* and pKpQIL-
270 98b (CP014765) from *K. pneumoniae*, both recently described in the United States, harboured deletions
271 located in the same region substituted in pKpQIL-D1. The system *vagCD* is thought to help plasmid
272 maintenance by preventing the cell division until plasmid replication is complete.[27] The loss of the
273 plasmid maintenance system in these pKpQIL-derivatives may have increased the chances of being
274 acquired by hosts in which their replication may be less efficient.

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

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

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Disclaimer

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314 **References**

315 1. Munoz-Price LS, Poirel L, Bonomo RA *et al*. Clinical epidemiology of the global expansion of
316 *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013; **13**: 785-96.
317 2. Canton R, Akova M, Carmeli Y *et al*. Rapid evolution and spread of carbapenemases among
318 Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012; **18**: 413-31.
319 3. Akpaka PE, Swanston WH, Ihemere HN *et al*. Emergence of KPC-producing *Pseudomonas*
320 *aeruginosa* in Trinidad and Tobago. *J Clin Microbiol* 2009; **47**: 2670-1.
321 4. Robledo IE, Aquino EE, Sante MI *et al*. Detection of KPC in *Acinetobacter* spp. in Puerto Rico.
322 *Antimicrob Agents Chemother* 2010; **54**: 1354-7.
323 5. Poirel L, Nordmann P, Lagrutta E *et al*. Emergence of KPC-producing *Pseudomonas aeruginosa* in
324 the United States. *Antimicrob Agents Chemother* 2010; **54**: 3072.
325 6. Villegas MV, Lolans K, Correa A *et al*. First identification of *Pseudomonas aeruginosa* isolates
326 producing a KPC-type carbapenem-hydrolyzing beta-lactamase. *Antimicrob Agents Chemother* 2007; **51**:
327 1553-5.
328 7. Wolter DJ, Khalaf N, Robledo IE *et al*. Surveillance of carbapenem-resistant *Pseudomonas*
329 *aeruginosa* isolates from Puerto Rican Medical Center Hospitals: dissemination of KPC and IMP-18 beta-
330 lactamases. *Antimicrob Agents Chemother* 2009; **53**: 1660-4.
331 8. Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-
332 risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* 2011; **35**: 736-55.
333 9. Pournaras S, Protonotariou E, Voulgari E *et al*. Clonal spread of KPC-2 carbapenemase-producing
334 *Klebsiella pneumoniae* strains in Greece. *J Antimicrob Chemother* 2009; **64**: 348-52.
335 10. Navon-Venezia S, Leavitt A, Schwaber MJ *et al*. First report on a hyperepidemic clone of KPC-3-
336 producing *Klebsiella pneumoniae* in Israel genetically related to a strain causing outbreaks in the United
337 States. *Antimicrob Agents Chemother* 2009; **53**: 818-20.
338 11. Leavitt A, Carmeli Y, Chmelnitsky I *et al*. Molecular epidemiology, sequence types, and plasmid
339 analyses of KPC-producing *Klebsiella pneumoniae* strains in Israel. *Antimicrob Agents Chemother* 2010;
340 **54**: 3002-6.
341 12. Giani T, Pini B, Arena F *et al*. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella*
342 *pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 2013;
343 **18**.
344 13. Kitchel B, Rasheed JK, Patel JB *et al*. Molecular epidemiology of KPC-producing *Klebsiella*
345 *pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob*
346 *Agents Chemother* 2009; **53**: 3365-70.
347 14. Palepou M, Woodford M, Hope R *et al*. Novel class A carbapenemase, KPC-4, in an *Enterobacter*
348 isolate from Scotland. In*: Abstracts of the Fifteenth European Congress of Clinical Microbiology and*
349 *Infectious Diseases, Copenhagen, 2005*. Abstract 1134_01_20: European Society of Clinical Microbiology
350 and Infectious Diseases, Basel, Switzerland.
351 15. Woodford N, Zhang J, Warner M *et al*. Arrival of *Klebsiella pneumoniae* producing KPC
352 carbapenemase in the United Kingdom. *J Antimicrob Chemother* 2008; **62**: 1261-4.
353 16. Poole K, George R, Decraene V *et al*. Active case finding for carbapenemase-producing
354 Enterobacteriaceae in a teaching hospital: prevalence and risk factors for colonization. *J Hosp Infect*
355 2016; **94**: 125-9.
356 17. Findlay J, Hopkins KL, Doumith M *et al*. KPC enzymes in the UK: an analysis of the first 160 cases
357 outside the North-West region. *J Antimicrob Chemother* 2016; **71**: 1199-206.

358 18. Leavitt A, Chmelnitsky I, Carmeli Y *et al*. Complete nucleotide sequence of KPC-3-encoding
359 plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. *Antimicrob Agents*
360 *Chemother* 2010; **54**: 4493-6.
361 19. Chavda KD, Chen L, Jacobs MR *et al*. Molecular Diversity and Plasmid Analysis of KPC-Producing
362 *Escherichia coli*. *Antimicrob Agents Chemother* 2016; **60**: 4073-81.
363 20. Chen L, Chavda KD, Melano RG *et al*. Comparative genomic analysis of KPC-encoding pKpQIL-like
364 plasmids and their distribution in New Jersey and New York Hospitals. *Antimicrob Agents Chemother*
365 2014; **58**: 2871-7.
366 21. Papagiannitsis CC, Di Pilato V, Giani T *et al*. Characterization of KPC-encoding plasmids from two
367 endemic settings, Greece and Italy. *J Antimicrob Chemother* 2016; **71**: 2824-30.
368 22. Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*
369 2001; **48 Suppl 1**: 5-16.
370 23. Yigit H, Queenan AM, Anderson GJ *et al*. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1,
371 from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001; **45**:
372 1151-61.
373 24. Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J*
374 *Microbiol Methods* 2005; **63**: 219-28.
375 25. Villa L, Garcia-Fernandez A, Fortini D *et al*. Replicon sequence typing of IncF plasmids carrying
376 virulence and resistance determinants. *J Antimicrob Chemother* 2010; **65**: 2518-29.
377 26. Alikhan NF, Petty NK, Ben Zakour NL *et al*. BLAST Ring Image Generator (BRIG): simple
378 prokaryote genome comparisons. *BMC Genomics* 2011; **12**: 402.
379 27. Pullinger GD, Lax AJ. A *Salmonella* Dublin virulence plasmid locus that affects bacterial growth
380 under nutrient-limited conditions. *Mol Microbiol* 1992; **6**: 1631-43.

381

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**	$\mathbf{IncFII_{K2}}$	**+**	**+**	**+**	**+**	**+**		**+**	**pKpQIL-like**
T5	*K. pneumoniae*	2009	258 (CG258)	E	3	a	IncFII_{K2}	+	+	+	+	+		+	pKpQIL-like
T6	***K. pneumoniae***	**2009**	**258 (CG258)**	**F**	**3**	**a**	$\mathbf{IncFII_{K2}}$	**+**	**+**	**+**	**+**	**+**		**+**	**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**	$\mathbf{IncFII_{K2}}$	**+**	**+**	**+**	**+**	**+**		**+**	**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**	$\mathbf{IncFII_{K2}}$	**+**	**+**	**+**	**+**	**+**		**+**	**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

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

385

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	

387

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

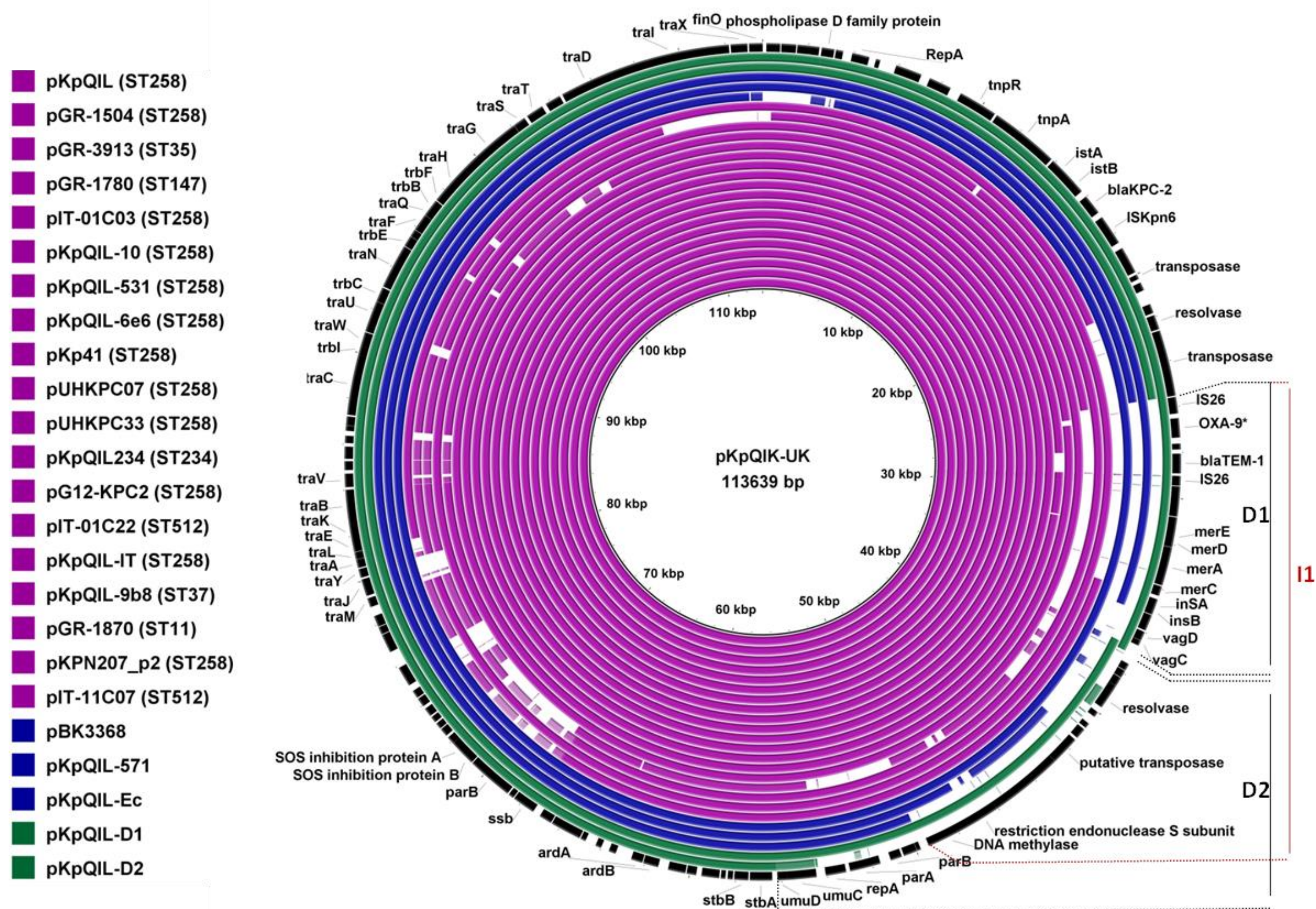
		Other centres						3	11	0	
	Other spp.	North-West England			2	1		2	3	0	
		Other centres					1		2	0	
Amikacin	*K. pneumoniae*	North-West England		5	6	1	2		5	73.7	70.5
		Other centres	1	1	1	1	3	1	6	50	
	Other spp.	North-West England		1	3	2	2			100	
		Other centres		2					1	66.7	
Gentamicin	*K. pneumoniae*	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.

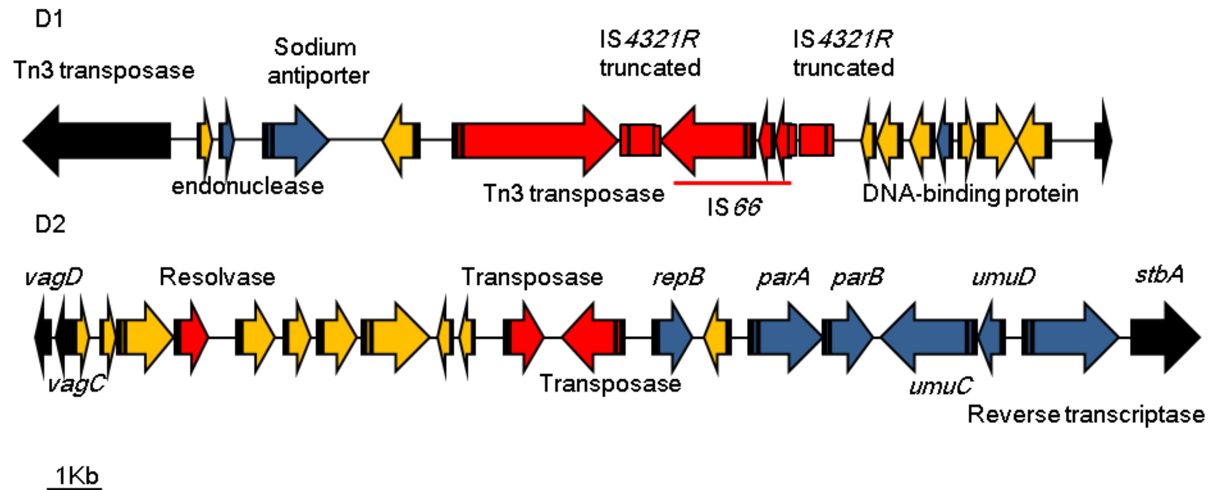
395 Figure 1. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC

396 A. Sequence homologies amongst published pKpQIL-like plasmids.



397

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



399

400

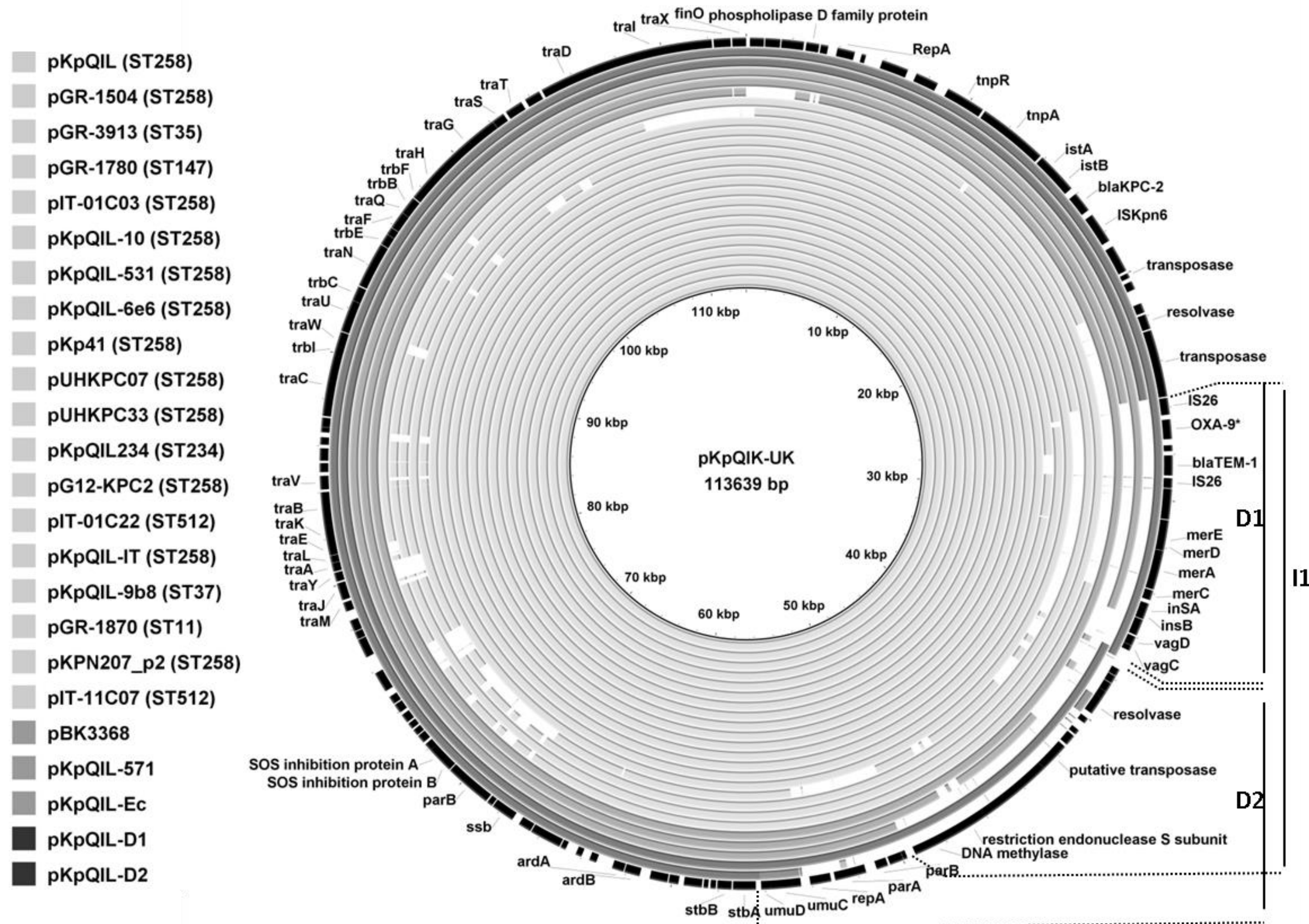
401 (A) Coloured rings showed published plasmids from *K. pneumoniae* (purple), *E. coli* (blue) and those generated in this study (green). D1 and D2
402 indicated the fragments of pKpQIL plasmid that were substituted in pKpQIL-D1 and pKpQIL-D2, respectively, while the pKpQIL fragment indicated I1
403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

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

406

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

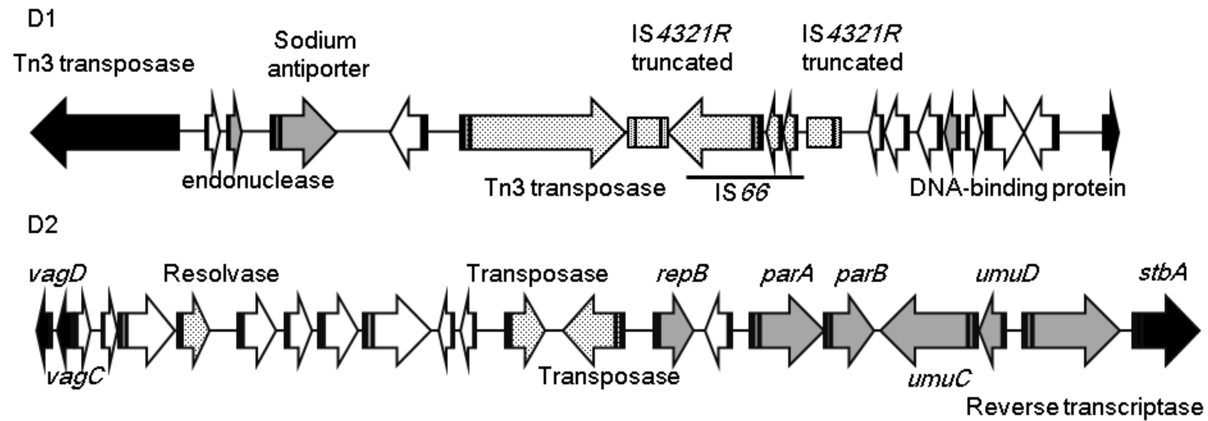
408 A. Sequence homologies amongst published pKpQIL-like plasmids.



409

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

411



412

413

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

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

420