

KPC carbapenemases in the United Kingdom: an analysis of the first 160 cases outside the NW region

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Running heading: KPC carbapenemases in the United Kingdom

Keywords: KPC; Enterobacteriaceae; carbapenem; UK, plasmids

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1 **Abstract**

2 **Objectives:** *Klebsiella pneumoniae* carbapenemases (KPCs) have been increasingly
3 reported in the UK since 2003. We analysed patient and isolate data for KPC-positive
4 bacteria confirmed by the national reference laboratory from UK laboratories, with the
5 exception of the North-West England region, where the epidemiology has previously been
6 studied, from August 2003 to August 2014.

7 **Methods:** MICs were determined by BSAC agar dilution methodology. Carbapenem-
8 resistant isolates lacking imipenem/EDTA synergy were tested by PCR for *bla*_{KPC}. Multi-
9 locus sequence typing and *bla*_{KPC} sequencing was performed on a subset of isolates.
10 Plasmid analysis was performed by transformation, PCR-based replicon typing and, in some
11 cases, whole-plasmid sequencing. Patient data provided by the sending laboratories were
12 reviewed.

13 **Results:** Two hundred and ten KPC-producing isolates were submitted from 71 UK
14 laboratories outside North-West England, representing 160 patients. All were
15 Enterobacteriaceae, predominantly *K. pneumoniae* (82%; 172/210), and most (91%;
16 191/210) were obtained from hospitalised patients. Analysis of 123 isolates identified *bla*_{KPC-2}
17 (64%; 79/123), *bla*_{KPC-3} (27%; 33/123) and *bla*_{KPC-4} (9%; 11/123). Within *K. pneumoniae*,
18 clonal group (CG) sequence type (ST) 258 was dominant (64%; 54/84), however 21
19 unrelated STs were also identified. Plasmid analysis identified a diverse range of plasmids of
20 at least 11 different replicon types, found in multiple STs and species.

21 **Conclusions:** KPC enzymes are increasingly detected in Enterobacteriaceae in the UK
22 outside North-West England, despite a lack of reported outbreaks. *K. pneumoniae* CG258
23 are the dominant hosts although plasmid spread also plays a significant role in dissemination
24 of KPCs between other *K. pneumoniae* STs and enterobacterial species.

25

26 Introduction

27 *Klebsiella pneumoniae* carbapenemases (KPCs) were first identified in 1996 in a *K.*
28 *pneumoniae* isolate obtained from a patient hospitalised in North Carolina, USA.¹ Since then
29 they have disseminated globally, predominantly among Enterobacteriaceae, although there
30 are also reports of production by *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolates
31 in the Americas.²⁻⁴ They have been reported in all inhabited continents, with numerous
32 outbreaks described, particularly in Greece, Israel, Italy, the USA and China.⁴ Many of these
33 outbreaks are associated with an internationally-disseminated lineage of *K. pneumoniae*,
34 sequence type (ST) 258, and other members of its clonal group (CG) CG258, which
35 comprises ST258, its single locus variants (SLVs), such as ST512, and their SLVs.⁵ KPCs
36 are typically found encoded within the Tn3-based transposon, Tn4401, of which there are
37 five isoforms (*a*, *b*, *c*, *d* and *e*) as defined by insertions or deletions within a polymorphic
38 region immediately upstream of the *bla*_{KPC} gene.⁶ The first fully-sequenced KPC-encoding
39 plasmid was an IncFIB/IncFII_k replicon type designated pKpQIL, from an ST258 isolate in
40 Israel, and highly similar plasmids have since been found in several other countries.⁷⁻⁹ KPC
41 has also been reported to be carried by plasmids of other replicon types including IncI2,
42 IncN, IncL/M and IncX, though these seem to be less frequent hosts of its gene.¹⁰⁻¹²

43 Most bacteria with KPC enzymes are multi-resistant, harbouring genes whose products
44 compromise non-β-lactam antibiotics (e.g. *aac(6′)-Ib*, encoding resistance to
45 aminoglycosides except gentamicin),^{13,14} resulting in a paucity of treatment options. The *K.*
46 *pneumoniae* ST258 lineage usually remains susceptible only to colistin, gentamicin and
47 tigecycline; however, there have been documented outbreaks of colistin-resistant *K.*
48 *pneumoniae* ST258, thereby further reducing therapeutic options.^{15,16}

49 The first KPC enzyme in the UK was identified in 2003 and was found in an *Enterobacter*
50 *cloacae* complex blood culture isolate from Scotland.¹⁷ The first *K. pneumoniae* ST258
51 isolate was found in a urine specimen in 2007,¹⁸ also in Scotland and since then, numbers of

52 KPC-producing isolates referred to Public Health England's (PHE) Antimicrobial Resistance
53 and Healthcare Associated Infections (AMRHAI) Reference Unit have risen sharply.⁴ Most
54 (>95%) originate from hospitals in North-West England (defined as the counties of Cheshire,
55 Cumbria, Greater Manchester, Lancashire and Merseyside) where an outbreak, centred in
56 Manchester, has been ongoing since 2010, despite control efforts.^{4,19} In contrast with most
57 international experience, this outbreak is polyclonal in nature and attributable to the
58 horizontal spread of a pKpQIL-like plasmid amongst multiple strains of multiple species of
59 Enterobacteriaceae.^{4,19} Similar polyclonal situations have been described recently in other
60 countries, including Spain and Canada.^{20,21}

61 Here we describe the first 160 bacteria producing KPC enzymes referred to AMRHAI from
62 infected or colonised UK patients outside the North-West of England.

63

64 **Materials and methods**

65 **Bacterial isolates, identification and susceptibility testing**

66 Isolates had been submitted to PHE's AMRHAI Reference Unit from laboratories across the
67 UK (excluding the North West) between August 2003 and 12th August 2014 for investigation
68 of 'unusual' resistance, including to carbapenems. They were identified using chromogenic
69 agars [CHROMagar™ Orientation (CHROMagar, Paris, France) and Brilliance UTI (Oxoid,
70 Basingstoke, UK)], API-20E tests (bioMérieux SA, Marcy-l'Etoile, France) or, since August
71 2012, by MALDI-ToF Mass Spectrometry (Bruker Microflex LT, Bruker Daltonik GmbH,
72 Bremen, Germany).

73 Antibiotic susceptibilities (MICs) were determined by the British Society for Antimicrobial
74 Chemotherapy (BSAC) agar dilution²² using AMRHAI's standard Gram-negative antibiotic
75 panel, which includes ertapenem, meropenem and imipenem (with/without 320 mg/L EDTA
76 to detect metallo-carbapenemase producers). MICs were interpreted using BSAC or
77 EUCAST breakpoints.^{23,24}

78

79 **Screening for KPC genes**

80 Isolates exhibiting raised cefotaxime and ceftazidime MICs with no significant clavulanic acid
81 synergy, and resistance, based on EUCAST/BSAC criteria to one or more of imipenem,
82 meropenem ertapenem, but lacking imipenem/EDTA synergy (≥ 8 fold potentiation of
83 imipenem by 320 mg/L EDTA) were screened by in-house PCR for KPC genes,¹ and/or with
84 a commercial microarray (Check-Points CT102, Check-Points, Wageningen, The
85 Netherlands).²⁵

86

87 **Whole Genome Sequencing (WGS)**

88 Genomes were sequenced using the Nextera sample preparation method and the standard
89 2 x 251-base sequencing protocols on a MiSeq instrument (Illumina, San Diego, CA, USA).
90 Reads were assembled into contigs using VelvetOptimiser
91 (<http://bioinformatics.net.au/software.velvetoptimiser.shtml>), with k-mer values from 55 to 75.
92 Sequence types and plasmid replicon types were extracted *in silico* by BLASTn using
93 reference sequences from (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>) and
94 <http://pubmlst.org/plasmid/> databases.

95

96 **Multi-locus sequence typing (MLST) and sequencing of KPC alleles**

97 A subset of 123 isolates (100 *K. pneumoniae*, 16 *E. cloacae* complex, four *Escherichia coli*,
98 two *K. oxytoca* and one *Citrobacter freundii*), geographically representative of submissions
99 and selected from throughout the study period, were chosen for further analysis. Sequence
100 types were determined for *K. pneumoniae*, *E. cloacae* complex and *E. coli* isolates by
101 traditional multi-locus sequence typing (MLST)²⁶⁻²⁸ or inferred from WGS data. The *bla*_{KPC}
102 alleles were defined either by sequencing PCR amplicons as previously described¹ or from
103 analysis of WGS data.

104

105 **Plasmid transformation, replicon typing and plasmid sequencing**

106 Transformation of plasmids encoding KPC enzymes was attempted by electroporation using
107 the subset of 123 isolates and *E. coli* Alpha-Select recipient cells (Bioline, London, UK).
108 Transformants were selected on LB agar containing 100 mg/L ampicillin, and colonies were
109 screened for *bla*_{KPC} by PCR. A subgroup of 59 transformants was selected and subjected to
110 WGS as above; selection was based on their geographical and temporal distribution, KPC
111 alleles, species of origin and STs.

112 Replicon typing of *bla*_{KPC} plasmids was performed as described previously^{29,30} or was
113 inferred from WGS data.

114

115 **Patient demographic information**

116 Patient data were obtained from the accompanying request forms sent with submissions
117 from referring laboratories. A patient was categorized as 'new' if they were found to have
118 KPC-positive isolates detected by AMRHAI for the first time and 'known' if KPC-positive
119 isolates, irrespective of species, had previously been identified from the patient by AMRHAI.

120

121 **Data analysis**

122 Data were analysed using Microsoft Excel and Bionumerics software v6.1 (Applied Maths,
123 Sint-Martens-Latem, Belgium).

124

125 **Results**

126 **Demographics of patients affected and distribution**

127 During the study period, AMRHAI confirmed 210 KPC-positive isolates from outside of
128 North-West England. These were submitted by 71 UK laboratories and obtained from 160
129 patients. Figure 1 illustrates the distribution of these isolates among 'new' and 'known'
130 patients, and among submitting laboratories. The first three KPC-producing organisms, as
131 previously reported,¹⁷ were *E. cloacae* isolates found in blood specimens from a single
132 patient across consecutive years (one in 2003 and two in 2004) from Scotland. All were
133 found to produce the KPC-4 variant. The first KPC-producing *K. pneumoniae* isolate was
134 identified in 2007 in a blood specimen, also from Scotland.¹⁸ The numbers of 'new' patients
135 increased significantly from 2008 onwards (Figure 1).

136 KPC-producing isolates were submitted from laboratories across all 11 UK regions studied.
137 The national distribution of affected patients, as ascertained by AMRHAI referrals, was as
138 follows: England (n=124), Scotland (n=26), Northern Ireland (n=9) and Wales (n=1). The
139 greatest number of affected patients was from the Yorkshire and the Humber region (n=39),
140 followed by London (n=27) and the West Midlands (n=20).

141 Most source patients were hospitalized (86%; 138/160), but a few were outpatients (4%;
142 6/160) or in primary care (6%; 10/160), or were from patients in an unknown setting (4%;
143 6/160). The mean patient age was 60.4 years and most were male (58%, 92/160).

144 Foreign travel history was available for 51/160 (32%) patients. Of these, 19 patients had
145 documented travel within the previous six months to: Greece (11/19), Italy (2/19), Bulgaria,
146 Curaçao, India, Israel, Macedonia, and Saudi Arabia (one patient each). Four of these 19,
147 with histories of travel to Curaçao, Greece, Israel and Italy, were known to have been
148 hospitalised whilst abroad. Information on patient transfers between UK regions was very
149 limited, however 5/160 (3%) patients were known to have had KPC-producing isolates
150 submitted from hospitals across two UK regions. Four KPC-positive patients had previously

151 been hospitalised in the North-West (Manchester) prior to KPC isolations in Wales, Northern
152 Ireland and Yorkshire and the Humber (two patients), and one KPC-positive patient had
153 previously been hospitalised in Yorkshire and the Humber prior to the East Midlands.

154 Single KPC-producing isolates were referred from 125/160 (78%) patients and multiple
155 isolates were referred from the remaining 35/160 (22%). Amongst the 35 patients with
156 multiple KPC-producing isolates, six (17%) yielded KPC-producing isolates of different
157 species or genera and 14 (40%) had KPC-producing isolates obtained from different
158 anatomical sites; the KPC-positive isolates were referred over a period of <14 days in 19/35
159 (54%) instances and over a period >6 months from just one patient.

160 The date when the sample was taken was available for 89% (187/210) of isolates and the
161 median duration between this date and the isolate being received at AMRHAI was 8 days
162 (Range = 1-49 days).

163

164 **Microbiology**

165 All KPC-positive isolates were Enterobacteriaceae. The majority were *K. pneumoniae* (82%;
166 173/210) followed by *E. cloacae* complex (11%; 24/210), *E. coli* (4%; 9/210), *K. oxytoca* (1%;
167 3/210) and *C. freundii* (<1%; 1/210).

168 If samples, rather than patients were considered as the denominator, most were taken in
169 hospitals (91%; 191/210), but some were from general practice urines (5%; 11/210) and a
170 few samples were from an unknown setting (4%; 8/210). The most frequently reported
171 specimen types were urines (33%; 70/210), followed by screening swabs (24%; 50/210).
172 Ten percent (21/210) of isolates were obtained from blood cultures or line tips, 13% (29/210)
173 from tissue and fluid samples and 10% (21/210) from faeces (Table 1).

174

175 **KPC alleles and typing of the isolates**

176 The KPC variants were defined for 59% (123/210) of isolates, distributed throughout the
177 entire collection period (2003-2014). Of these, 64% (79/123) were *bla*_{KPC-2}, 27% (33/123)
178 were *bla*_{KPC-3}, and 9% (11/123) were *bla*_{KPC-4}; no other variants were detected. Isolates
179 harbouring *bla*_{KPC-2} were geographically scattered and included *K. pneumoniae*, *E. cloacae*
180 complex, and *E. coli*. *bla*_{KPC-3}-positive isolates were also geographically scattered, but were
181 all *K. pneumoniae*. *bla*_{KPC-4} was found only in *E. cloacae* complex isolates from Scotland.

182

183 *Klebsiella pneumoniae*

184 One hundred of the 173 *K. pneumoniae* isolates were typed by MLST. After the exclusion of
185 isolates exhibiting the same ST from single patients, 84 results remained for analysis.
186 Almost two-thirds (64%; 54/84) belonged to the CG258, comprising isolates belonging to
187 ST258 (n=41), ST512 (n=9), ST11 (n=3) and ST833 (n=1). Between 2007 and 2014, CG258
188 isolates were submitted from 31 laboratories across all UK regions studied (10/11) except
189 Wales; 54% (22/41) produced KPC-2 and the remaining 46% (19/41) produced KPC-3
190 enzymes. One of the earliest ST258 isolates was obtained in 2008 and was from the urine of
191 a patient previously hospitalised in Israel;¹⁸ another ST258 isolate was from a wound swab
192 of a patient hospitalised in Greece in 2011. However, the first ST258 isolate, from 2007,
193 came from a patient that had no foreign travel history. ST512 isolates were referred from six
194 laboratories between 2008 and 2014 from three UK regions, and all produced KPC-3. One
195 patient who had *K. pneumoniae* ST512 isolated from a sputum sample in 2012 had been
196 hospitalised in Italy within the previous six months. ST11 isolates were submitted between
197 2009 and 2011 from two laboratories in two UK regions. One patient, with an ST11 isolate
198 from urine, had a history of travel to Curaçao where he had been hospitalised for two weeks
199 and had undergone urinary catheterisation. The last member of the CG258, a single isolate
200 of ST833, produced KPC-2 and the patient had no known history of travel. There were four

201 outbreaks in hospitals across three regions caused by members of CG258; ST11 producing
202 KPC-2 in 2 patients, ST512 producing KPC-3 in 3 patients, ST258 producing KPC-3 in 2
203 patients, and ST258 producing KPC-2 in 3 patients. These outbreaks were over periods
204 ranging from 1 week to 2 months.

205 Thirty (36%) characterised isolates did not belong to CG258. Of these, five isolates belonged
206 to ST661, were submitted from two laboratories, located in different regions between July
207 2013 and May 2014 and produced KPC-2 enzyme. The remaining 25, from 16 laboratories,
208 represented 20 different STs and produced either KPC-2 (24/25) or KPC-3 (1/25) (Figure 2).

209

210 *Enterobacter cloacae* complex

211 Sixteen of 24 *E. cloacae* complex isolates were typed by MLST. After the exclusion of
212 isolates exhibiting the same sequence type from a single patient, 12 results remained for
213 analysis. Eight isolates that were submitted from five laboratories in Scotland between 2003
214 and 2013, belonged to ST171 and produced KPC-4 enzyme. The remaining four isolates
215 were ST133 (two isolates from the same hospital), ST190 and ST56 (one isolate each), and
216 all produced KPC-2.

217

218 *Escherichia coli*

219 Four of the nine *E. coli* isolates were typed by MLST and represented four unrelated
220 sequence types: ST12, ST127, ST131 and ST744. All four produced the KPC-2 enzyme.

221

222 **Antibiotic susceptibility**

223 The MIC distributions of KPC-positive isolates are shown in Table 2. All isolates were
224 resistant to ertapenem and most were resistant or non-susceptible to imipenem (98%;

225 202/207) and meropenem (97%; 201/208). All meropenem MICs were above the EUCAST
226 screening concentration (MICs >0.125 mg/L). All members of the *K. pneumoniae* CG258
227 were resistant to tobramycin and most were also non-susceptible or resistant to amikacin
228 (89%; 57/64), however two-thirds were susceptible to gentamicin. By comparison, members
229 of non-CG258 *K. pneumoniae* STs (n=36) were more often susceptible to all three
230 aminoglycosides (61%, 86% and 64% were susceptible to tobramycin, amikacin and
231 gentamicin respectively). Most non-*K. pneumoniae* isolates also were susceptible to all three
232 aminoglycosides. All members of the CG258 were resistant or non-susceptible to
233 ciprofloxacin, compared with 47% (17/36) of other *K. pneumoniae* STs and 62% (23/37) of
234 all other species. Colistin resistance was observed in 26 *K. pneumoniae* isolates, of which
235 belonged to CG258, three to three unrelated STs; the STs of the remaining 10 colistin-
236 resistant isolates were undetermined. Colistin MICs for resistant isolates ranged from 4-32
237 mg/L (Table 2) and resistant isolates originated from laboratories/hospitals across six UK
238 regions. Susceptibility to tigecycline was observed in 61% (125/205) of all isolates but only in
239 48% (31/64) of CG258 isolates.

240

241 **Plasmid analysis**

242 Transformants expressing KPC enzymes were obtained for 90/123 (73%) of the subset of
243 isolates chosen for further analysis. PCR-based replicon typing and whole genome
244 sequencing were performed on 90/90 and 59/90 transformants, respectively. The data
245 revealed the following replicon types; IncFIB/IncFII_k (n=49), IncN (n=17), IncFII_k (n=8),
246 IncFIB (n=3), IncR (n=3), ColE-like (n=2), IncI2 (n=2), IncFIA (n=1), IncP-6 (n=1), IncX3
247 (n=1), and three plasmids were of untypable replicon types. Most (80%; 39/49) plasmids with
248 the IncFIB/IncFII_k replicon type were obtained from members of the CG258. Of the 59 that
249 were sequenced, 30/35 IncFIB/IncFII_k plasmids exhibited >99% sequence identity to
250 pKpQIL (GenBank Accession No. NC_014016).

251 The sizes of the sequenced KPC plasmids ranged from ~13kb to ~224kb. In all sequenced
252 plasmids the KPC genes were located within Tn4401 isoforms *a*, *b*, *c* or *d* (Table 3). All
253 plasmids encoded variants *bla*_{KPC-2} or *bla*_{KPC-3} with the exception of the two ColE-like
254 plasmids found in *E. cloacae* complex isolates from Scotland, which encoded *bla*_{KPC-4}. Most
255 of the plasmid replicon types were recovered from multiple UK regions (Table 3). Some KPC
256 plasmids were also shown to carry a number of additional antibiotic resistance genes (Table
257 3).

258

259 **Discussion**

260 This report reviews the first 160 recorded patients infected or colonised by KPC-positive
261 bacteria in the UK, excluding the North West of England (which accounts for most cases), as
262 ascertained from referrals to PHE's AMRHAI Reference Unit. Isolates were submitted over
263 an eleven-year period from August 2003 to August 2014, from laboratories across the UK.

264 KPC-producing isolates were submitted from all UK regions over this study period. The
265 majority of isolates were obtained from clinical specimens (133/210), and these were
266 predominantly urine samples (70/133). All isolates were multi-resistant to antibiotics and
267 exhibited non-susceptibility to at least one of the three carbapenems tested. The only
268 antibiotics that retained relatively good levels of activity *in vitro* were colistin (87%),
269 gentamicin (65%) and tigecycline (61%). The use of colistin as monotherapy against KPC-
270 producers has limitations due to its nephrotoxicity and neurotoxicity and also the danger of
271 selecting for colistin-resistant mutants.³¹ The potential for expansion of colistin-resistant
272 variants is evidenced by reports of outbreaks caused by colistin-resistant members of the
273 ST258 clone.^{15,16} In this study we found 26 *K. pneumoniae* isolates that were resistant to
274 colistin, most of these were members of CG258, and they were found in 10/11 UK regions.
275 The use of tigecycline is limited by its inability to achieve high concentrations in the urine and
276 blood, and is licensed for the treatment of complicated skin and skin structure infections, and
277 complicated intra-abdominal infections.³² Several antibiotic combinations have been used for
278 the treatment of infections caused by KPC-producing bacteria including: colistin with
279 aminoglycosides/carbapenems/fluoroquinolones, tigecycline with aminoglycosides, and
280 several beta-lactam and fluoroquinolone/aminoglycoside combinations.^{31,33} Such
281 combination therapies have been shown to be more effective than monotherapy and are
282 believed to reduce the likelihood of the development of resistant mutants.^{31,33}

283 Although travel history was available for just one-third of the patients, 11/51 had travelled to
284 Greece in the previous six months, two had travelled to Italy and one had travelled to Israel,

285 all of which have reported nationwide KPC outbreaks within their hospitals.⁴ One patient with
286 a wound infection caused by *K. pneumoniae* ST258 had previously been hospitalised in
287 Greece, where ST258 lineages have caused multiple outbreaks since 2007.⁴ Another patient
288 had been hospitalised in Curaçao for a period of two weeks prior to isolation of KPC-positive
289 *K. pneumoniae* in the UK.³⁴ The KPC-2-producing *K. pneumoniae* ST11 isolated from his
290 urine was most likely acquired in Curaçao, where he was catheterised.^{34,35} One of the
291 patients who had travelled to and been hospitalised in Italy was found to have a KPC-3-
292 producing *K. pneumoniae* ST512 (CG258), which is reported to be a problematic clone in
293 Italy, causing outbreaks in several hospitals.^{4,36} Whilst it is not possible to know conclusively
294 where acquisition of the KPC-producing bacteria took place, it is clear that international
295 travel continues to play a significant role in the importation of KPC-producing clones, and
296 this has been illustrated in the worldwide spread of members of CG258.⁴

297 The finding that four patients had KPC isolations in hospitals across two UK regions
298 demonstrates that domestic travel and patient transfers may play a vital role in the
299 dissemination of KPC-producing bacteria within the UK. This has the potential to be
300 particularly problematic when one UK region has an ongoing outbreak (the North-West) and
301 could conceivably result in the expansion of this outbreak.

302 At the time of this study there were 22 known KPC variants (KPC-2 – KPC-23) identified
303 (www.lahey.org/studies/) and only three variants were found here: KPC-2, KPC-3 and KPC-
304 4. KPC-2 and KPC-3 are the most common variants worldwide, and their genes are often
305 harboured on pKpQIL and pKpQIL-like plasmids.^{9,37} We first identified KPC-4 in 2003, and
306 this variant has recently also been found in the USA on IncL/M plasmids in both *E. cloacae*
307 and *S. marcescens*, and encoded by an IncN plasmid in *K. pneumoniae*.^{10,11} In this study all
308 of the KPC-4-producing isolates were *E. cloacae* complex ST171 and had been isolated in
309 five laboratories in Scotland over a 10-year period (from 2003 to 2013). Sequencing
310 identified ~13 kb ColE-like plasmids encoding *bla*_{KPC-4} in two of these isolates. Despite the

311 long-term persistence of this KPC-4-producing clone, it has not caused recognised
312 outbreaks and its KPC-encoding plasmid has not spread to other hosts.

313 Although the worldwide dissemination of KPC-producing bacteria is substantially associated
314 with a single clonal group (*K. pneumoniae* CG258), KPC enzymes have been found in
315 numerous other *K. pneumoniae* sequence types and in other bacterial species.^{4,10} *bla*_{KPC}
316 have been recorded as carried by several plasmids of different incompatibility groups,
317 including IncF, IncI2, IncN, IncL/M and IncX.^{10-12,37} Here we found KPC genes in four
318 bacterial species and in 34 different sequence types, carried by at least 11 plasmid replicon
319 types, suggesting that both plasmid spread and the mobility between plasmids plays an
320 important role in the dissemination of KPC in the UK. Within CG258 alone we found at least
321 8 different KPC plasmid replicon types, indicative of the success of this clonal group as a
322 host of KPC plasmids. The observation that most plasmids were of the IncFIB/IncFII_K and
323 highly homologous to pKpQIL show that pKpQIL-like plasmids are dominant in the UK.

324 There are numerous reports of outbreaks of KPC-producers from other countries,^{4,8,12,15}
325 associated particularly with members of CG258. We have shown here that *K. pneumoniae*
326 ST258 is the dominant host of KPC enzymes in the UK outside of North-West England and
327 that multiple UK hospitals have been challenged by the introduction of this successful clone
328 and its close relatives since 2007. Nevertheless, to date there have been very few clusters of
329 infections or colonisations caused by *K. pneumoniae* ST258 in the UK. Whether the lack of
330 CG258 dissemination can be attributed to better screening and/or compliance with infection
331 control practices in the UK is unknown, but this does underline the need for continued
332 surveillance and for implementation of rigorous infection prevention and control measures.³⁸

333

334 **Acknowledgements**

335

336 **Funding**

337

338 **Transparency declaration**

339 PHE's AMRHAI Reference Unit has received financial support for conference attendance,
340 lectures, research projects or contracted evaluations from numerous sources, including:
341 Achaogen Inc, Allecra Antiinfectives GmbH, Amplex, AstraZeneca UK Ltd, Becton Dickinson
342 Diagnostics, The BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department
343 of Health, Food Standards Agency, GlaxoSmithKline Services Ltd, Henry Stewart Talks,
344 IHMA Ltd, Merck Sharpe & Dohme Corp, Meiji Seika Kiasya Ltd, Momentum Biosciences
345 Ltd, Nordic Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Rokitan
346 Ltd, Smith & Nephew UK Ltd, Trius Therapeutics, VenatoRx and Wockhardt Ltd.

347 **DML:** Advisory Boards or ad hoc consultancy – Achaogen, Adenium, Alere, Allecra, Astellas,
348 AstraZeneca, Basilea, Bayer, BioVersys, Cubist, Curetis, Cycle, Discuva, Forest, GSK, Meiji,
349 Pfizer, Roche, Shionogi, Tetrphase, VenatoRx, Wockhardt; Paid lectures – AOP Orphan,
350 Astellas, AstraZeneca, Bruker, Curetis, Merck, Pfizer, Leo; shareholdings in– Dechra, GSK,
351 Merck, Perkin Elmer, Pfizer amounting to <10% of portfolio value.

352

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463 enterobacteriaceae-early-detection-management-and-control-toolkit-for-acute-
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Hospital Setting									
Species	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces	Not known	GP urines	Total
<i>C. freundii</i>	0	1	0	0	0	0	0	0	1
<i>E. cloacae</i> complex	7	0	4	2	4	2	0	3	22
<i>E. coli</i>	4	1	0	0	0	2	0	1	8
<i>Klebsiella</i> spp.	44	47	17	12	24	16	4	7	171
Total	55	49	21	14	28	20	4	11	202
Unknown Setting									
Species	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces	Not known		Total
<i>E. cloacae</i> complex	1	1	0	0	0	0	0		2
<i>E. coli</i>	1	0	0	0	0	0	0		1
<i>Klebsiella</i> spp.	2	1	0	0	1	0	1		5
Total	4	2	0	0	1	0	1		8

Table 1. Source and species for the KPC-positive isolates from different settings.

Species/ST	Antibiotic (range tested, mg/L)	EUCAST breakpoints ≤S/>R	Number of Isolates with MIC (mg/L)											NA	%S			
			≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128					
<i>K. pneumoniae</i> /CG258 (n=64)	Ertapenem (0.125-16)	≤0.5/>1										64 ^a				0		
<i>K. pneumoniae</i> /other STs (n=36)													36 ^a				0	
<i>K. pneumoniae</i> /NT (n=73)											2		69 ^a			2	0	
<i>K. oxytoca</i> /NT (n=3)													3 ^a				0	
<i>E. cloacae</i> complex (n=24)										1	4	3	16 ^a				0	
<i>E. coli</i> (n=9)											1	2	6 ^a				0	
<i>C. freundii</i> (n=1)													1 ^a				0	
Total										1	5	7	195 ^a			2	0	
<i>K. pneumoniae</i> /CG258 (n=64)	Imipenem (0.06-128)	≤2/>8									6	13	15	19	11	0		
<i>K. pneumoniae</i> /other STs (n=36)							1				1	20	5	1	7	1	3	
<i>K. pneumoniae</i> /NT (n=73)											1	11	23	18	11	7	2	0
<i>K. oxytoca</i> /NT (n=3)													3					0
<i>E. cloacae</i> complex (n=24)						1	1	2			5	1	8	3	1	2		13
<i>E. coli</i> (n=9)												5	4					0
<i>C. freundii</i> (n=1)														1				0
Total						1	1	3			6	24	71	42	32	27	3	2
<i>K. pneumoniae</i> /CG258 (n=64)	Meropenem (0.06-32)	≤2/>8									1	5	5	53 ^a			0	
<i>K. pneumoniae</i> /other STs (n=36)												5	12	19 ^a			0	
<i>K. pneumoniae</i> /NT (n=73)												2	10	21	38 ^a		2	0
<i>K. oxytoca</i> /NT (n=3)														3				0
<i>E. cloacae</i> complex (n=24)						1	1	4	1		1	10	2	4 ^a				29
<i>E. coli</i> (n=9)											3	4	1	1				0
<i>C. freundii</i> (n=1)														1				0
Total						1	1	4	1		7	34	44	116 ^a			2	3
<i>K. pneumoniae</i> /CG258 (n=64)	Amikacin (0.5-64)	≤8/>16			1 ^b	1			2	3		11	36	10 ^a			11	
<i>K. pneumoniae</i> /other STs (n=36)					1 ^b	12	11		3	4		4	1					86
<i>K. pneumoniae</i> /NT (n=73)					1 ^b	11	10		9	9		9	16	6 ^a			2	55
<i>K. oxytoca</i> /NT (n=3)												3						100
<i>E. cloacae</i> complex (n=24)						2 ^b	10	8		3	1							100

Total				132 ^b	47	2	2	7	1	16 ^a	3	87
<i>K. pneumoniae</i> /CG258 (n=64)				4	27	27	6					48
<i>K. pneumoniae</i> /other STs (n=36)			3 ^b	12	12	8	1					75
<i>K. pneumoniae</i> /NT (n=73)				11	29	20	4	4	3 ^a		2	56
<i>K. oxytoca</i> /NT (n=3)	Tigecycline		1 ^b	2								100
<i>E. cloacae</i> complex (n=24)	(0.25-16)	≤1/>2		7	9	2	1	2			3	76
<i>E. coli</i> (n=9)			6 ^b	1	1	1						89
<i>C. freundii</i> (n=1)						1						0
Total			10 ^b	37	78	59	12	6	3 ^a		5	61

S, susceptible; R, resistant; NA, not available; NT, not typed; CG, clonal group; ST, sequence type.

Cells highlighted in dark grey are resistant; those in light grey are intermediate; and white are susceptible.

^aMIC greater than or equal to indicated value.

^bMIC less than or equal to the indicated value.

Table 2. MIC distributions for KPC-producing isolates (n=210).

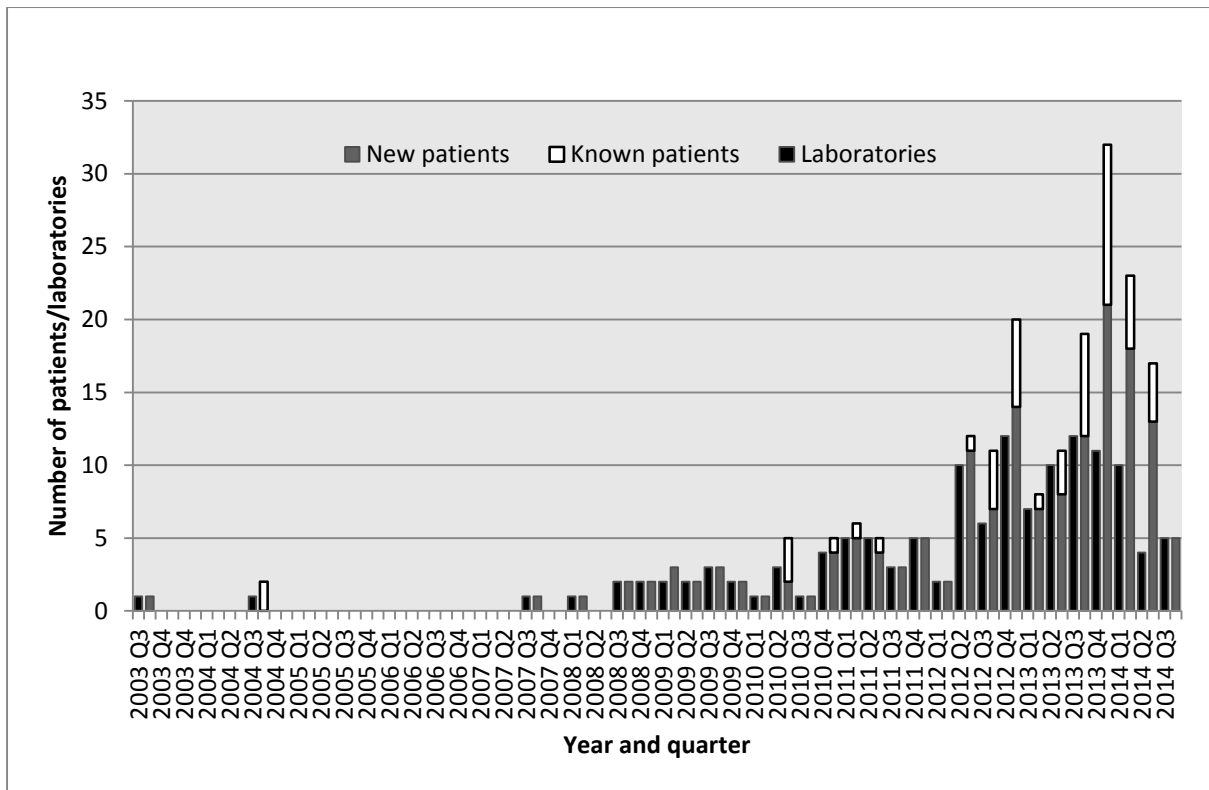


Figure 1. Numbers of new and known affected patients and laboratories sending KPC-positive isolates per quarter during the study period.

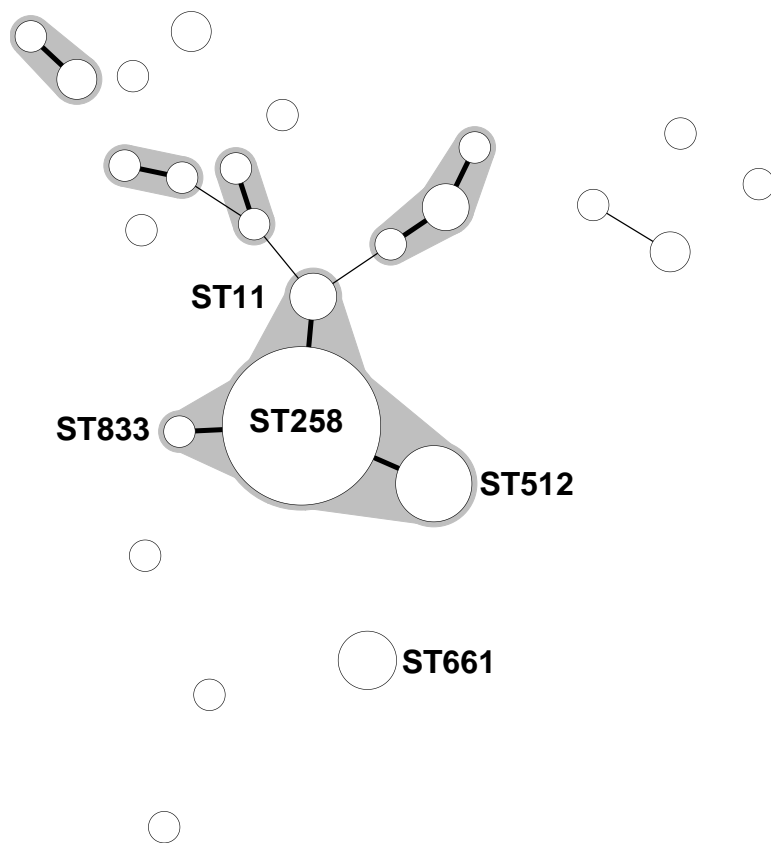


Figure 2. Minimum spanning tree of the MLST profiles of 84 KPC-positive *K. pneumoniae* isolates, received between September 2007 and July 2014 from 65 submitting laboratories. The shaded areas represent members of the ST258 clonal group. Members of the ST258 clonal group are labelled, as are other STs with >4 submissions. The diameter of the circle represents the number of isolates of that particular ST. Thick solid lines represent single-locus variants; thin solid lines represent double-locus variants, and the absence of connecting lines indicates multi-locus variants

Replicon Types	No. of Plasmids	Approx. Size (kb)	Species	KPC Variants	Other resistance genes	STs* carrying plasmids	No. of Regions	Tn4401 Isoform(s)
ColE-like	2	13	<i>E. cloacae</i> complex	KPC-4		171	1	a
IncFIA	1	52	<i>K. pneumoniae</i>	KPC-2	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-9}	15	1	a
IncFIB	1	76	<i>K. pneumoniae</i>	KPC-3	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-9}	258	1	a
IncFII _K	3	97 - 213	<i>K. pneumoniae</i>	KPC-2	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-9} , <i>aadA2</i> , <i>aadA5</i> , <i>dfrA12</i> , <i>dfrA17</i> , <i>catA1</i> , <i>sul</i> , <i>mph(A)</i> , <i>qnrB1</i>	258/321/1162	3	a
IncFIB/IncFII _K	35	106 - 224	<i>K. pneumoniae</i>	KPC-2/3	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-9} , <i>aadA2</i> , <i>aadA5</i> , <i>dfrA12</i> , <i>dfrA17</i> , <i>catA1</i> , <i>sul</i> , <i>mph(A)</i> , <i>qnrB1</i>	15/147/252/258/307/321/512/678/709/732/896/1162/1163	7	a
IncI2	2	77	<i>K. pneumoniae</i>	KPC-3	<i>bla</i> _{OXA-9} , <i>bla</i> _{TEM-1} , <i>aac(6')-Ib</i> , <i>aadA1</i>	258	1	b
IncN	8	59 - 76	<i>K. pneumoniae</i> / <i>K. oxytoca</i>	KPC-2	<i>bla</i> _{TEM-1} , <i>bla</i> _{TEM-135} , <i>aph(6)-Ic</i> , <i>sul</i> , <i>dfrA</i> , <i>qnrB2</i>	258/336/1026	4	b/c
IncR	3	48 - 69	<i>K. pneumoniae</i>	KPC-2/3	<i>aac(6')-Ib</i> , <i>aadA2</i> , <i>catA1</i> , <i>cmlA1</i> , <i>mef(B)</i>	258	2	a/b
IncX3	1	53	<i>K. pneumoniae</i>	KPC-3	<i>bla</i> _{TEM-1} , <i>qnrB2</i> , <i>aph(6)-Ic</i> , <i>sul</i> , <i>dfrA</i>	258	1	a
IncP-6	1	38	<i>K. pneumoniae</i>	KPC-2	<i>bla</i> _{TEM-33}	11	1	a
Untypable	2	62 - 89	<i>K. pneumoniae</i>	KPC-2/3	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-9} , <i>aph(6)-Ic</i> , <i>aac(6')-Ib</i> , <i>aadA1</i> , <i>qnrB2</i> , <i>aph(6)-Ib</i> , <i>sul</i> , <i>dfrA</i>	258/833	2	b/d

Table 3. The features of 59 KPC plasmids sequenced.*STs were determined for *K. pneumoniae* and *E. cloacae* complex isolates.