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

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Abstract

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

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

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

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

Introduction

Klebsiella pneumoniae carbapenemases (KPCs) were first identified in 1996 in a *K. pneumoniae* isolate obtained from a patient hospitalised in North Carolina, USA.[1] Since then they have disseminated globally, predominantly among Enterobacteriaceae, although there are also reports of production by *Acinetobacter* spp. and *Pseudomonas aeruginosa* isolates in the Americas.[2-4] They have been reported in all inhabited continents, with numerous outbreaks described, particularly in Greece, Israel, Italy, the USA and China.[4] Many of these outbreaks are associated with an internationally-disseminated lineage of *K. pneumoniae*, sequence type (ST) 258, and other members of its clonal group (CG) CG258, which comprises ST258, its single locus variants (SLVs), such as ST512, and their SLVs.[5] KPCs are typically found encoded within the Tn*3*-based transposon, Tn*4401*, of which there are five isoforms (*a*, *b*, *c*, *d* and *e*) as defined by insertions or deletions within a polymorphic region immediately upstream of the bla_{KPC} gene.[6] The first fully-sequenced KPC-encoding plasmid was an IncFIB/IncFII$_K$ replicon type designated pKpQIL, from an ST258 isolate in Israel, and highly similar plasmids have since been found in several other countries.[7-9] KPC has also been reported to be carried by plasmids of other replicon types including IncI2, IncN, IncL/M and IncX, though these seem to be less frequent hosts of its gene.[10-12]

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

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

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

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

Materials and methods

Bacterial isolates, identification and susceptibility testing

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

Antibiotic susceptibilities (MICs) were determined by the British Society for Antimicrobial Chemotherapy (BSAC) agar dilution[22] using AMRHAI's standard Gram-negative antibiotic panel, which includes ertapenem, meropenem and imipenem (with/without 320 mg/L EDTA to detect metallo-carbapenemase producers). MICs were interpreted using BSAC or EUCAST breakpoints.[23,24]

Screening for KPC genes

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

Whole Genome Sequencing (WGS)

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

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

A subset of 123 isolates (100 *K. pneumoniae*, 16 *E. cloacae* complex, four *Escherichia coli*, two *K. oxytoca* and one *Citrobacter freundii*), geographically representative of submissions and selected from throughout the study period, were chosen for further analysis. Sequence types were determined for *K. pneumoniae*, *E. cloacae* complex and *E. coli* isolates by traditional multi-locus sequence typing (MLST)[26-28] or inferred from WGS data. The bla_{KPC} alleles were defined either by sequencing PCR amplicons as previously described[1] or from analysis of WGS data.

Plasmid transformation, replicon typing and plasmid sequencing

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

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

Patient demographic information

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

Data analysis

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

Results

Demographics of patients affected and distribution

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

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

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

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

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

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

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

Microbiology

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

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

KPC alleles and typing of the isolates

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

Klebsiella pneumoniae

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

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

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

Enterobacter cloacae complex

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

Escherichia coli

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

Antibiotic susceptibility

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

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

Plasmid analysis

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

The sizes of the sequenced KPC plasmids ranged from ~13kb to ~224kb. In all sequenced plasmids the KPC genes were located within Tn*4401* isoforms *a*, *b*, *c* or *d* (Table 3). All plasmids encoded variants bla_{KPC-2} or bla_{KPC-3} with the exception of the two ColE-like plasmids found in *E. cloacae* complex isolates from Scotland, which encoded bla_{KPC-4}. Most of the plasmid replicon types were recovered from multiple UK regions (Table 3). Some KPC plasmids were also shown to carry a number of additional antibiotic resistance genes (Table 3).

Discussion

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

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

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

all of which have reported nationwide KPC outbreaks within their hospitals.[4] One patient with a wound infection caused by *K. pneumoniae* ST258 had previously been hospitalised in Greece, where ST258 lineages have caused multiple outbreaks since 2007.[4] Another patient had been hospitalised in Curaçao for a period of two weeks prior to isolation of KPC-positive *K. pneumoniae* in the UK.[34] The KPC-2-producing *K. pneumoniae* ST11 isolated from his urine was most likely acquired in Curaçao, where he was catheterised.[34,35] One of the patients who had travelled to and been hospitalised in Italy was found to have a KPC-3-producing *K. pneumoniae* ST512 (CG258), which is reported to be a problematic clone in Italy, causing outbreaks in several hospitals.[4,36] Whilst it is not possible to know conclusively where acquisition of the KPC-producing bacteria took place, it is clear that international travel continues to play a significant role in the importation of KPC-producing clones, and this has been illustrated in the worldwide spread of members of CG258.[4]

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

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

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

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

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

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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
C. freundii	0	1	0	0	0	0	0	0	1
E. cloacae complex	7	0	4	2	4	2	0	3	22
E. coli	4	1	0	0	0	2	0	1	8
Klebsiella 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
E. cloacae complex	1	1	0	0	0	0	0	2
E. coli	1	0	0	0	0	0	0	1
Klebsiella 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)												
			≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	NA	%S
K. pneumoniae/CG258 (n=64)	Ertapenem (0.125-16)	≤0.5/>1								64[a]					0
K. pneumoniae/other STs (n=36)										36[a]					0
K. pneumoniae/NT (n=73)									2	69[a]				2	0
K. oxytoca/NT (n=3)										3[a]					0
E. cloacae complex (n=24)							1	4	3	16[a]					0
E. coli (n=9)								1	2	6[a]					0
C. freundii (n=1)										1[a]					0
Total							1	5	7	195[a]				2	0
K. pneumoniae/CG258 (n=64)	Imipenem (0.06-128)	≤2/>8							6	13	15	19	11		0
K. pneumoniae/other STs (n=36)							1		1	20	5	1	7	1	3
K. pneumoniae/NT (n=73)								1	11	23	18	11	7	2	0
K. oxytoca/NT (n=3)										3					0
E. cloacae complex (n=24)					1	1	2	5	1	8	3	1	2		13
E. coli (n=9)									5	4					0
C. freundii (n=1)											1				0
Total					1	1	3	6	24	71	42	32	27	3	2
K. pneumoniae/CG258 (n=64)	Meropenem (0.06-32)	≤2/>8						1	5	5	53[a]				0
K. pneumoniae/other STs (n=36)									5	12	19[a]				0
K. pneumoniae/NT (n=73)								2	10	21	38[a]			2	0
K. oxytoca/NT (n=3)										3					0
E. cloacae complex (n=24)				1	1	4	1	1	10	2	4[a]				29
E. coli (n=9)								3	4	1	1				0
C. freundii (n=1)											1				0
Total				1	1	4	1	7	34	44	116[a]			2	3
K. pneumoniae/CG258 (n=64)	Amikacin (0.5-64)	≤8/>16			1[b]	1		2	3	11	36	10[a]			11
K. pneumoniae/other STs (n=36)					1[b]	12	11	3	4	4	1				86
K. pneumoniae/NT (n=73)					1[b]	11	10	9	9	9	16	6[a]		2	55
K. oxytoca/NT (n=3)								3							100
E. cloacae complex (n=24)					2[b]	10	8	3	1						100

E. coli (n=9)						1	3	2	1	2					78
C. freundii (n=1)									1						100
Total					5[b]	35	32	22	19	26	53	16[a]		2	54
K. pneumoniae/CG258 (n=64)	Gentamicin (0.125-32)	≤2/>4			4	16	23	6	7	3	5[a]				67
K. pneumoniae/other STs (n=36)				3	16	4			2	1	10[a]				64
K. pneumoniae/NT (n=73)				2	15	18	6		4	1	25[a]			2	58
K. oxytoca/NT (n=3)						1	2								100
E. cloacae complex (n=24)				5	11	3	1				4[a]				83
E. coli (n=9)					2	2	2	1			2[a]				67
C. freundii (n=1)											1[a]				0
Total				10	48	44	34	7	13	5	47[a]			2	65
K. pneumoniae/CG258 (n=64)	Tobramycin (0.125-32)	≤2/>4								9	55[a]				0
K. pneumoniae/other STs (n=36)				3	15	3	1	1		5	8[a]				61
K. pneumoniae/NT (n=73)				3	15	5	1			7	40[a]			2	34
K. oxytoca/NT (n=3)						2		1							67
E. cloacae complex (n=24)				3	12	4	1			1	3[a]				83
E. coli (n=9)					2	1	4				2[a]				78
C. freundii (n=1)											1[a]				0
Total				9	44	15	7	2		22	108[a]			2	36
K. pneumoniae/CG258 (n=64)	Ciprofloxacin (0.125-8)	≤0.5/>1							64[a]						0
K. pneumoniae/other STs (n=36)			15[b]	1	3	3			14[a]						53
K. pneumoniae/NT (n=73)			15[b]	1	1	3	2	2	47[a]					2	24
K. oxytoca/NT (n=3)			3[b]												100
E. cloacae complex (n=24)			1[b]	1	4	2	1	2	13[a]						25
E. coli (n=9)			2[b]	2	1				4[a]						56
C. freundii (n=1)									1						0
Total			36[b]	5	9	8	3	4	143[a]					2	7
K. pneumoniae/CG258 (n=64)	Colistin (0.5-32)	≤2/>2			38[b]	11	1		3		10[a]			1	79
K. pneumoniae/other STs (n=36)					21[b]	12			2	1					92
K. pneumoniae/NT (n=73)					50[b]	10	1	2	2		6[a]			2	86
K. oxytoca/NT (n=3)					2[b]	1									100
E. cloacae complex (n=24)					18[b]	6									100
E. coli (n=9)					3[b]	6									100
C. freundii (n=1)						1									100

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

[a]MIC greater than or equal to indicated value.

[b]MIC less than or equal to the indicated value.

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

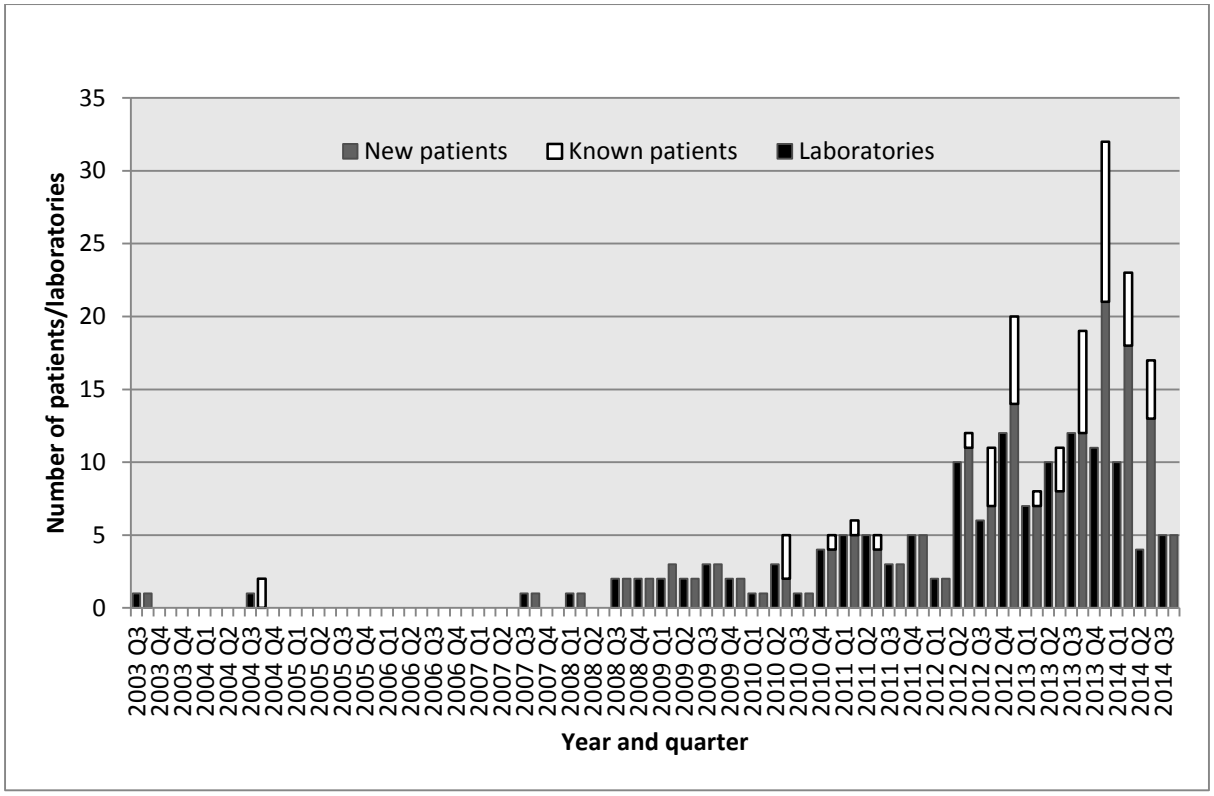

Figure 1. Numbers of new and known affected patients and laboratories sending KPC-positive isolates per quarter during the study period.

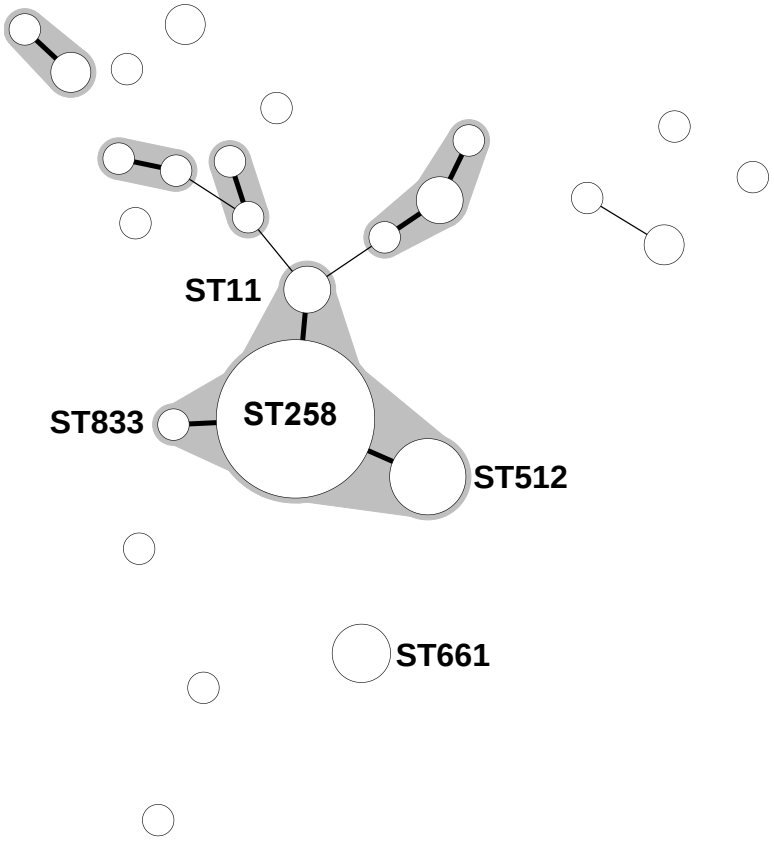

Figure 2. Minimum spanning tree of the MLST profiles of 84 KPC-positive *K. pneumoniae* isolates, received between September 2007 and July 2014 from 65 submitting laboratories. The shaded areas represent members of the ST258 clonal group. Members of the ST258 clonal group are labelled, as are other STs 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	*E. cloacae* complex	KPC-4		171	1	a
IncFIA	1	52	*K. pneumoniae*	KPC-2	bla_{TEM-1}, bla_{OXA-9}	15	1	a
IncFIB	1	76	*K. pneumoniae*	KPC-3	bla_{TEM-1}, bla_{OXA-9}	258	1	a
$IncFII_K$	3	97 - 213	*K. pneumoniae*	KPC-2	bla_{TEM-1}, bla_{OXA-9}, *aadA2*, *aadA5*, *dfrA12*, *dfrA17*, *catA1*, *sul*, *mph*(A), *qnrB1*	258/321/1162	3	a
$IncFIB/IncFII_K$	35	106 - 224	*K. pneumoniae*	KPC-2/3	bla_{TEM-1}, bla_{OXA-9}, *aadA2*, *aadA5*, *dfrA12*, *dfrA17*, *catA1*, *sul*, *mph*(A), *qnrB1*	15/147/252/258/307/321/512/678/709/732/896/1162/1163	7	a
IncI2	2	77	*K. pneumoniae*	KPC-3	bla_{OXA-9}, bla_{TEM-1}, *aac(6')-Ib*, *aadA1*	258	1	b
IncN	8	59 - 76	*K. pneumoniae/ K. oxytoca*	KPC-2	bla_{TEM-1}, $bla_{TEM-135}$, *aph(6)-Id*, *sul*, *dfrA*, *qnrB2*	258/336/1026	4	b/c
IncR	3	48 - 69	*K. pneumoniae*	KPC-2/3	*aac(6')-Ib*, *aadA2*, *catA1*, *cmlA1*, *mef*(B)	258	2	a/b
IncX3	1	53	*K. pneumoniae*	KPC-3	bla_{TEM-1}, *qnrB2*, *aph(6)-Id*, *sul*, *dfrA*	258	1	a
IncP-6	1	38	*K. pneumoniae*	KPC-2	bla_{TEM-33}	11	1	a
Untypable	2	62 - 89	*K. pneumoniae*	KPC-2/3	bla_{TEM-1}, bla_{OXA-9}, *aph(6)-Id*, *aac(6')-Ib*, *aadA1*, *qnrB2*, *aph(6)-Ib*, *sul*, *dfrA*	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.