A Large, Refractory Nosocomial Outbreak of *Klebsiella pneumoniae* Carbapenemase-Producing *Escherichia coli* Demonstrates Carbapenemase Gene Outbreaks Involving Sink Sites Require Novel Approaches to Infection Control

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ABSTRACT Carbapenem-resistant *Enterobacteriaceae* (CRE) represent a health threat, but effective control interventions remain unclear. Hospital wastewater sites are increasingly being highlighted as important potential reservoirs. We investigated a large *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Escherichia coli* outbreak and wider CRE incidence trends in the Central Manchester University Hospital NHS Foundation Trust (CMFT) (United Kingdom) over 8 years, to determine the impact of infection prevention and control measures. Bacteriology and patient administration data (2009 to 2017) were linked, and a subset of CMFT or regional hospital KPC-producing *E. coli* isolates (*n* = 268) were sequenced. Control interventions followed international guidelines and included cohorting, rectal screening (*n* = 184,539 screens), environmental sampling, enhanced cleaning, and ward closure and plumbing replacement. Segmented regression of time trends for CRE detections was used to evaluate the impact of interventions on CRE incidence. Genomic analysis (*n* = 268 isolates) identified the spread of a KPC-producing *E. coli* outbreak clone (strain A, sequence type 216 [ST216]; *n* = 125) among patients and in the environment, particularly on 2 cardiac wards (wards 3 and 4), despite control measures. ST216 strain A had caused an antecedent outbreak and shared its KPC plasmids with other *E. coli* lineages and *Enterobacteriaceae* species. CRE acquisition incidence declined af-

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ter closure of wards 3 and 4 and plumbing replacement, suggesting an environmental contribution. However, ward 3/ward 4 wastewater sites were rapidly recolonized with CRE and patient CRE acquisitions recurred, albeit at lower rates. Patient relocation and plumbing replacement were associated with control of a clonal KPC-producing *E. coli* outbreak; however, environmental contamination with CRE and patient CRE acquisitions recurred rapidly following this intervention. The large numbers of cases and the persistence of bla_{KPC} in *E. coli*, including pathogenic lineages, are of concern.

KEYWORDS antimicrobial resistance, carbapenemase-producing *Enterobacteriaceae*, genome sequencing, infection control, molecular epidemiology

Carbapenem-resistant *Enterobacteriaceae* (CRE) represent a global public health threat (1). Major carbapenemases include the metallo-β-lactamases, some oxacillinases, and the *Klebsiella pneumoniae* carbapenemase (KPC) (encoded by bla_{KPC}), one of the most common carbapenemases globally (2). Transfer of carbapenemase genes on mobile genetic elements has resulted in rapid interspecies dissemination of carbapenem resistance (3, 4). Since few therapeutic options remain for CRE infections (5, 6), effective control is critical.

Escherichia coli is a major human pathogen, but it also a gastrointestinal commensal and can be transmitted between humans and the environment. Carbapenem resistance in *E. coli*, including that encoded by bla_{KPC}, is increasing (7, 8) but is uncommon, and KPC-producing *E. coli* outbreaks have not been observed to date. The emergence and persistence of carbapenem resistance in *E. coli* in human and/or environmental reservoirs are of concern.

CRE detections in England have increased since 2008 (9) and are approximately 10 times the national average in Greater Manchester (10). Central Manchester University Hospital NHS Foundation Trust (CMFT) has experienced an ongoing, multispecies, bla_{KPC}-associated CRE outbreak since 2009. Intensive infection prevention and control (IPC) measures, in line with national and international recommendations (11–13), have been implemented in response.

In 2015, a sudden increase in cases of fecal colonization with KPC-producing *E. coli* was detected in the Manchester Heart Centre (MHC) at the Manchester Royal Infirmary (part of CMFT). We retrospectively investigated the genomic epidemiology and evidence for nosocomial transmission of KPC-producing *E. coli* and KPC plasmids isolated from patients and the environment in this context, and we assessed the impact of guideline-compliant IPC bundles on CRE and KPC-producing *E. coli* incidence.

RESULTS

High prevalence of CRE colonization in the MHC. Between 1 April 2014 and 30 December 2014, 23 new CRE-colonized individuals were detected in the MHC, including 2 with *E. coli* (Fig. 1A). A CRE outbreak was declared on 2 January 2015, when 6 new CRE-colonized individuals were identified (4 with bla_{KPC} and 2 with bla_{NDM}; no *E. coli*). Consequently, intensified IPC measures were implemented (Fig. 1B; also see Table S1 in the supplemental material), and wards 3 and 4 were closed (on 6 January 2015), terminally cleaned (with hypochlorite), and decontaminated (with hydrogen peroxide vapor). Ward 3 was reopened on 11 January 2015, and ward 4 was reopened on 23 January 2015. High-risk patients (with previously detected CRE or a history of hospitalization abroad or in a UK hospital with known CRE transmission in the past 12 months) were screened; CRE-positive patients were transferred to a cohort ward or, if they required cardiac monitoring, to side rooms.

By January 2015, CMFT was operating a Trustwide CRE screening program (>110 screens/day) (Table S2). Between 1 September 2014 and 30 December 2014, screening transitioned from culture-based methods to PCR-based methods; during this period, 16,612 samples from 7,239 inpatients were screened using either culture (9,808 samples) or PCR and culture (6,804 samples), with an overall CRE prevalence of 3.8% (438
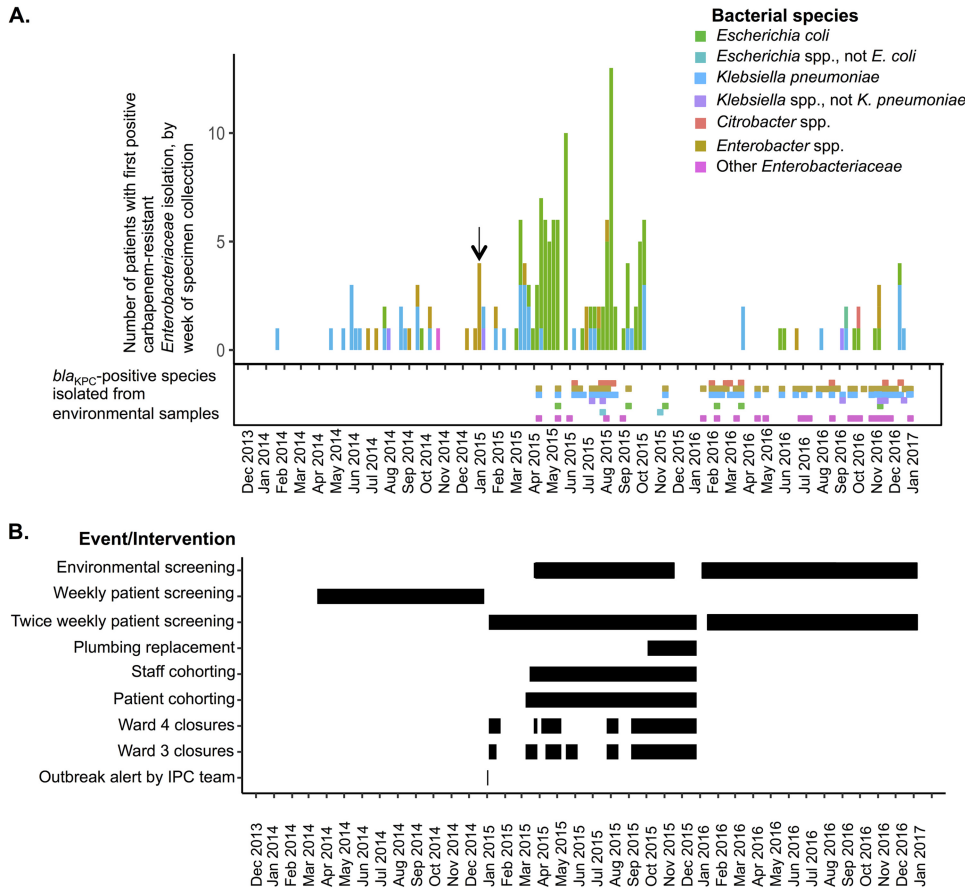
FIG 1 (A) Numbers of individuals in MHC wards with first CRE-positive detection, by week, stratified by genus group and species of the organism isolated. The bla_{KPC}-positive *Enterobacteriaceae* strains detected in environmental samples over the same time frame are also shown. The MHC outbreak was declared by the IPC team in the first week in 2015 (arrow). (B) Timeline of IPC measures instituted.

positive samples from 272 patients). Molecular mechanism data for 135/163 PCR-positive samples (83%) indicated that bla_{KPC} accounted for most carbapenem resistance (97%).

KPC-producing *E. coli* outbreak despite IPC interventions. Following the implementation of enhanced IPC activity, there was a further sharp increase in the number of CRE-colonized patients detected from 9 March 2015 (carbapenem-resistant [CR] *E. coli* and other species, mostly containing bla_{KPC} and a few with bla_{NDM}) (Fig. 1A). Ward 3 was again closed to admissions (from 11 March 2015 to 28 March 2015), and environmental decontamination was repeated; the following week, ward 4 was closed after detection of additional CRE-colonized patients (Fig. 1A and B). From 1 April 2015, KPC-producing *E. coli* predominated in the outbreak (Fig. 1A).

From April to September 2015, wards 3 and 4 were closed repeatedly, with 2 peaks in KPC-producing *E. coli* patient colonization (in April to May and in August) (Fig. 1B). Ward 3 capacity was reduced to 10 day-case beds (on 12 August 2015; day-case patients were not screened for CRE) and ward 4 capacity to 12 inpatient beds. Between 10 August 2015 and 28 September 2015, there were 27 new KPC-producing *E. coli* colonizations detected in the MHC (Fig. 1A) and 2 cases with other KPC-producing *Enterobacteriaceae* species. Of 88 KPC-producing *E. coli* cases between 24 February 2015 and 28 September 2015, 86 (98%) represented colonizations only; 1 individual additionally had a urinary tract infection and 1 a sternal wound infection (treated with gentamicin and ciprofloxacin, respectively, to which the isolates were susceptible).

CR *E. coli* cases in CMFT. CR *E. coli* had been isolated in CMFT prior to the 2015 MHC outbreak, with 514 CR *E. coli* cases (considering first positive results by patient from
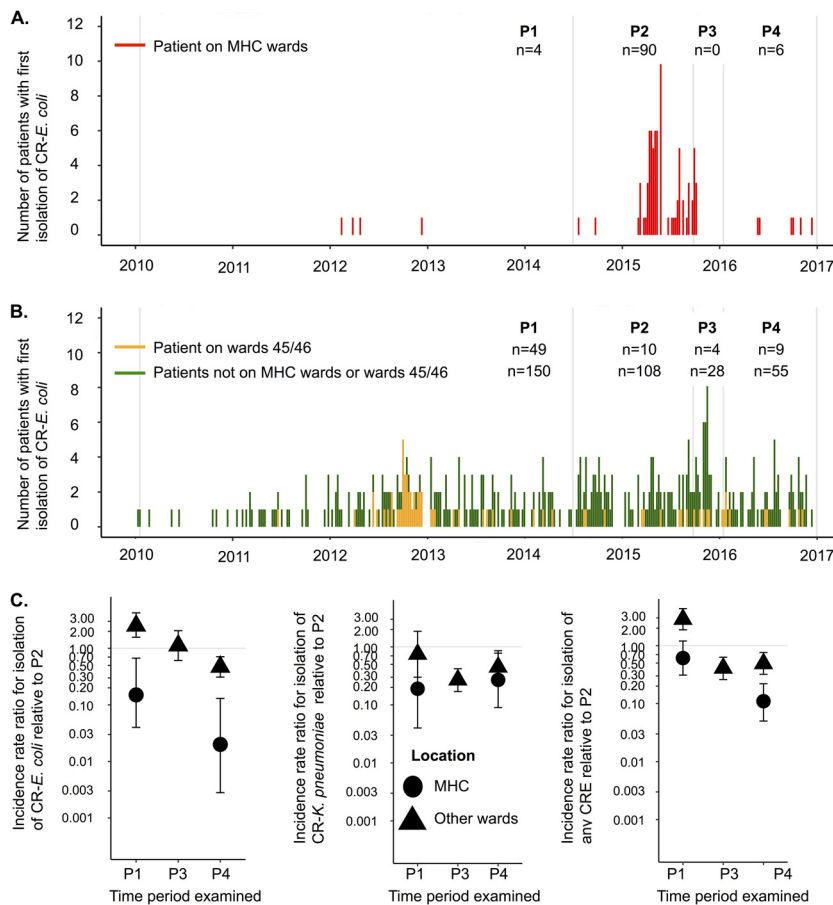
FIG 2 (A and B) Counts of individuals with first CR *E. coli* detection by ward location. Detections on days 0 and 1 of admission are excluded. Faint vertical lines correspond to the boundaries of the 4 time periods, as follows: period 1 (P1), prior to implementation of a systematic CPE rectal screening policy; period 2 (P2), implementation of a CPE rectal screening policy consistent with national guidance; period 3 (P3), closure of wards 3 and 4 and replacement of plumbing infrastructure; period 4 (P4), reopening of wards 3 and 4 to patient admissions. (C) Incidence rate ratios for rates of first positive CR *E. coli* detection, carbapenem-resistant *K. pneumoniae* detection, and any CRE detection ≥2 days postadmission, relative to period 2 in the same location (MHC versus the rest of CMFT). An incidence rate ratio is not shown for period 3 in the MHC due to unit closure during this period, to facilitate plumbing replacement.

clinical/screening isolates) in 2010 to 2016 (inclusive), including a separate outbreak on the gerontology wards (wards 45 and 46) in late 2012 (Fig. 2A and B). Of those, 434 cases were detected on day ≥2 of admission and 80 on day 0 or 1 of admission. Case peaks were not related to screening policy changes or rates (Fig. S6). CR *E. coli* strains were detected almost invariably from rectal screening samples (420/434 cases [97%]).

Environmental sampling yielding CRE from sinks and drains. Intermittent environmental sampling was undertaken to identify potential reservoirs. Overall, 927 samples from 833 sites were obtained between 9 April and 17 November 2015; 355 samples (38%) from 333 sites (40%) were from ward 3 or ward 4, and the remainder were from 11 other wards. A total of 850 samples were from sink/drain/shower/bath sites, 18 from toilets, hoppers, or sluices, and 33 from high-touch sites (including keyboards, door handles, and sponges; the labeling was unclear for 26 samples). Eighty-five samples (9%) and 72 sites (9%) were CRE positive, including 26/355 samples (7%) from 21/333 sites (6%) in wards 3 and 4. CRE-positive sites included shower drains ($n = 19$), sink taps ($n = 7$), sink drain tailpieces ($n = 10$), sink drain strainers ($n = 8$), sink trap water ($n = 1$), toilet bowls ($n = 1$), and other sites ($n = 26$). Common isolates cultured included *Klebsiella* spp. ($n = 34$), *Enterobacter* spp. ($n = 25$), and *E. coli* ($n = 11$) (Fig. 1A). All CRE-positive cultures were from wastewater/plumbing-associated sites; no other sites tested were CRE positive.

TABLE 1 Incidence rate ratios for detection from screening swabs ≥2 days after admission (a proxy marker of acquisition) in CMFT for all CRE cases, CR *E. coli* cases, and CR *K. pneumoniae* cases, modeling the impact of the ward 3 and ward 4 closures and plumbing replacement on acquisition

Location and period[a]	All CRE (3,086 cases)		CR *E. coli* (502 cases)		CR *K. pneumoniae* (1,134 cases)	
	IRR (95% CI)	P	IRR (95% CI)	P	IRR (95% CI)	P
MHC						
Week 3, 2010, to week 26, 2014 (period 1)	0.61 (0.31–1.20)	0.15	0.15 (0.04–0.67)	0.012	0.19 (0.04–0.82)	0.026
Week 27, 2014, to week 39, 2015 (period 2; reference period)	1.00		1.00		1.00	
Week 40, 2015, to week 2, 2016 (period 3; wards 3 and 4 closed)						
Week 3, 2016, to week 52, 2016 (period 4)	0.11 (0.05–0.22)	<0.001	0.02 (0.00–0.14)	<0.001	0.27 (0.09–0.78)	0.015
Other hospital locations						
Week 3, 2010, to week 26, 2014 (period 1)	2.85 (1.87–4.34)	<0.001	2.51 (1.57–4.03)	<0.001	0.75 (0.30–1.86)	0.53
Week 27, 2014, to week 39, 2015 (period 2; reference period)	1.00		1.00		1.00	
Week 40, 2015, to week 2, 2016 (period 3)	0.41 (0.26–0.63)	<0.001	1.12 (0.61–2.05)	0.71	0.27 (0.17–0.42)	<0.001
Week 3, 2016, to week 52, 2016 (period 4)	0.49 (0.32–0.76)	0.002	0.47 (0.31–0.71)	<0.001	0.47 (0.28–0.77)	0.003
MHC vs other location in reference period (period 2)	1.69 (0.81–3.50)	0.16	9.05 (3.98–20.55)	<0.001	0.45 (0.24–0.86)	0.015
Heterogeneity for reduction in MHC vs other location						
Week 3, 2010, to week 26, 2014 (period 1)		<0.001		0.001		0.098
Week 40, 2015, to week 2, 2016 (period 3)						
Week 3, 2016, to week 52, 2016 (period 4)		<0.001		0.003		0.31

[a]Four time periods were evaluated, as follows: period 1, prior to implementation of a systematic CPE rectal screening policy; period 2, implementation of a CPE rectal screening policy consistent with national guidance; period 3, closure of wards 3 and 4 and replacement of plumbing infrastructure; period 4, reopening of wards 3 and 4 to patient admissions. Period 2 was chosen as the reference period because of the change in screening policies between period 1 and period 2 (see Table S2 and Fig. S6 in the supplemental material), meaning that a greater incidence would be expected in period 2, due to more patients being screened every week. IRR, incidence rate ratio; CI, confidence interval.

Of 10 sites yielding 11 KPC-producing *E. coli* isolates, 5 were in the ward 3/ward 4 kitchen (14 to 18 May 2015 [*n* = 4] and 10 September 2015 [*n* = 1]), 1 was a ward 4 staff sink (14 May 2015), and 4 were kitchen sinks or drains on wards 31 and 32 (sampling in response to a separate ward 31/32 outbreak, 12 to 17 November 2015). Ward 3/ward 4 sink-specific interventions included sink trap replacement for CRE-colonized sinks (16 April 2015, 31 July 2015, and 11 August 2015) and horizontal pipework cleaning with a brush to try to remove biofilms (11 August 2015).

Cardiac service relocation and decline in CRE colonization incidence. Given the ongoing difficulty in preventing KPC-producing *E. coli* acquisitions and the isolation of KPC-producing *E. coli* from sink and drain sites, wards 3 and 4 were closed from 25 September 2015 and patients were relocated to another ward to allow replacement of the plumbing infrastructure back to the central drainage stacks. Replaceable sink plughole devices designed to prevent water aerosolization in the sink U-bend and to limit biofilm formation (HygieneSiphon; Aquafree) were installed.

Controlling for screening and compared to the period immediately before intervention (when screening policies were the same), the incidence of first detection of any CRE or *E. coli* strain decreased significantly following the plumbing intervention, both in the MHC and elsewhere in the hospital (Fig. 2C and Table 1). The decline in incidence was significantly greater in the MHC (heterogeneity *P* < 0.001), where incidence fell by 89% for any CRE strain and by 98% for CR *E. coli*. The incidence of CR *K. pneumoniae* also decreased significantly in both settings, but there was no evidence that the declines differed between the two settings (heterogeneity *P* = 0.31) (Table 1). However, when patients were transferred back to wards 3 and 4 (from 18 January 2016), CR *E. coli* continued to be detected in patients (6 first detections in 2016) (Fig. 2A). Patient colonization with other CRE strains was also observed, in numbers similar to those for 2014 (Fig. 1A); environmental contamination with CRE in sink and wastewater sites recurred rapidly (Fig. 1A), and 2 environmental sites (both ward utility room sink drains) were CRE positive even prior to patient readmissions to the ward, suggesting residual contamination after the plumbing replacement or reintroduction following the plumbing replacement but prior to patient readmissions.

Genomic epidemiology of KPC-producing *E. coli*. A total of 268 clinical and environmental CR *E. coli* isolates were sequenced, including 82 isolates from the MHC
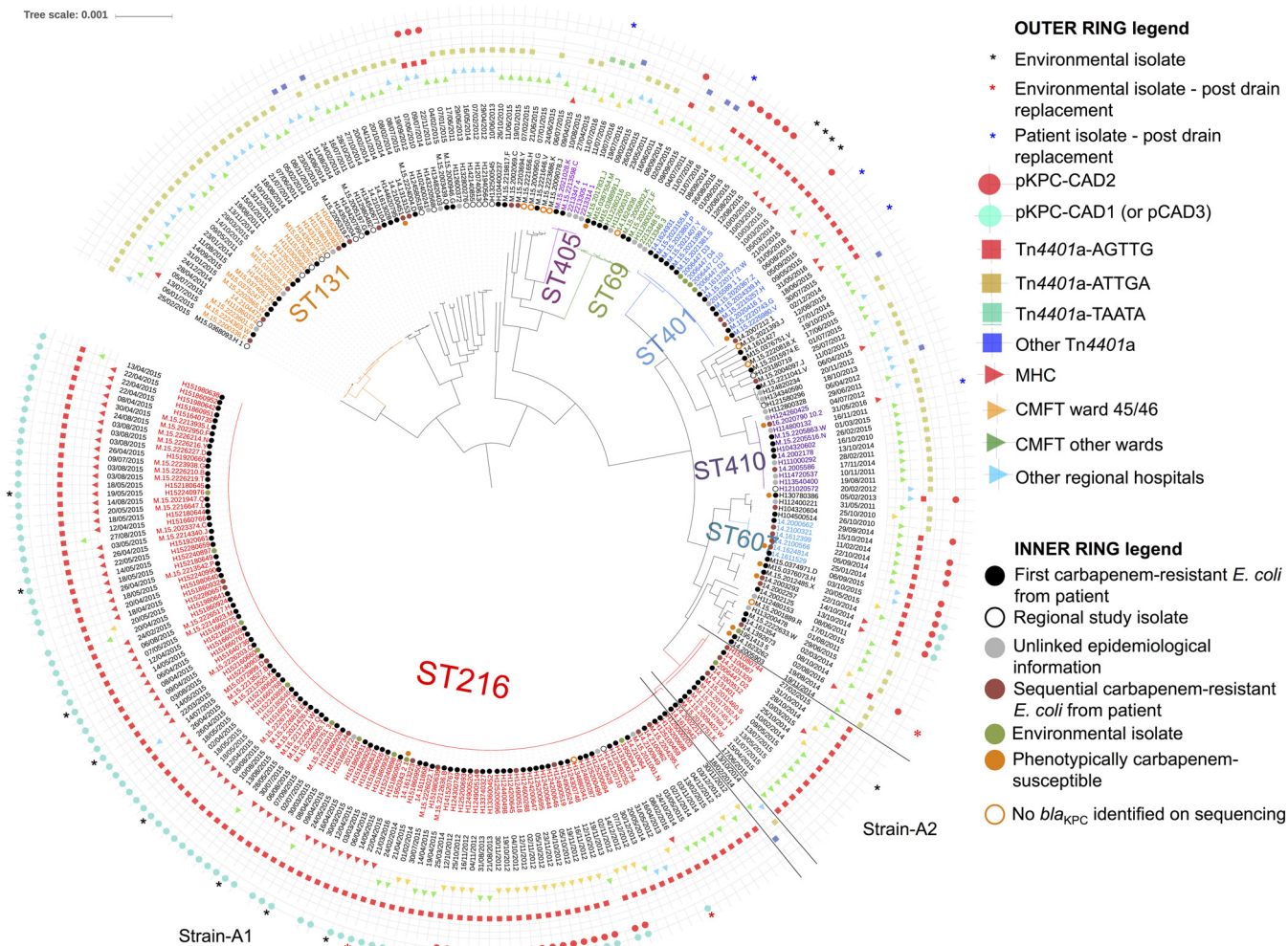
FIG 3 Recombination-corrected phylogeny of 259 sequenced KPC-producing *E. coli* isolates (and 9 *E. coli* isolates that were *bla*$_{KPC}$ negative on sequencing) from CMFT and other regional hospitals in northwest England, annotated with collection date, ward/center location, Tn*4401* type, and outbreak plasmid types. The earliest available sequences per patient are denoted "first carbapenem-resistant *E. coli* from patient" if the stored isolate collection date was ≤7 days from the first isolation date in the TRACE database or "sequential carbapenem-resistant *E. coli* from patient" if the stored isolate date was after that. KPC-producing *E. coli* isolates from a Public Health England (PHE) project that sequenced the first 10 KPC-producing *Enterobacteriaceae* strains from hospitals in northwest England (2009 to 2014) are denoted "regional study isolate." "Environmental isolate" denotes KPC-producing *E. coli* strains cultured during an initial environmental prevalence survey on wards 3 and 4 (10 March 2015), any KPC-producing *E. coli* strain isolated as part of subsequent, intermittent, IPC-associated environmental sampling (9 April 2015 to 17 November 2015), and isolates available at the time of analysis from environmental and patient samples from a separate ongoing study (commenced January 2016).

(2015 to 2016 [16 environmental isolates]), 36 from wards 45 and 46 (2010 to 2016), 109 from other CMFT wards or units, and 41 from other regional hospitals (Table S3). Nine isolates were *bla*$_{KPC}$ negative on sequencing; 5 of those isolates contained *bla*$_{OXA-48}$, 1 *bla*$_{OXA-181}$, and 1 *bla*$_{NDM-5}$, with no known carbapenem resistance mechanisms identified for the remaining 2. The 259 KPC-producing *E. coli* isolates included all 16 environmental CR *E. coli* isolates, 158 isolates that were the first CR *E. coli* isolates cultured from patients, and 38 sequentially cultured CR *E. coli* isolates from patients (longitudinal cultures from 12 patients); sequencing and patient epidemiological identifiers could not be linked for 47/259 isolates.

Forty sequence types (STs), including known pathogenic lineages (e.g., ST131), occurred among the KPC-producing *E. coli* isolates (Fig. 3; also see Table S3), highlighting regional KPC-producing *E. coli* diversity. In contrast, 67/80 MHC isolates (84%) were ST216, compared with 59/179 (33%) elsewhere. ST216 has rarely been reported in other settings.

ST216 KPC-producing *E. coli*. The ST216 KPC-producing *E. coli* group ($n = 126$, including 1 bla_{KPC}-negative isolate [H134880341]; 9,118 variable sites) was represented by 2 main genetic subgroups, consisting of 112 isolates (the main outbreak strain [strain A1 in Fig. 3]; ≤65 single-nucleotide variations [SNVs] among isolates in this cluster [2012 to 2016]) and 12 isolates (the secondary outbreak strain [strain A2 in Fig. 3]; ≤25 SNVs among isolates in this cluster and >7,800 SNVs divergent from strain A1 isolates [2012 to 2015]). Although the SNV-based distances between strains A1 and A2 were large, review of the ClonalFrameML output suggested that these differences represented a single "mega-recombination event" affecting ~1 Mb of the genome (Fig. S7).

All except 3 ST216 isolates carried bla_{KPC-2} in a Tn*4401*a transposon (14), which is typically associated with high-level bla_{KPC} expression (15), flanked by a 5-bp target site duplication, AGTTG, which was previously observed only with the Tn*4401*b isoform in an isolate from Colombia (Fig. 3; also see Table S3). This relatively unique transposon-flanking sequence unit was also observed in other lineages within CMFT (e.g., ST401) (Fig. 3). However, plasmid and resistance gene profiles varied considerably, even to some extent within the ST216 KPC-producing *E. coli* outbreak strains (Fig. 3; also see Fig. S8). Overall, these results demonstrated clonal expansion of specific KPC-producing *E. coli* strains, with significant accessory genome mobility. Most notable were the emergence and persistence of ST216 KPC-producing *E. coli* strain A1, which was isolated from patients and the environment over 4 years and caused outbreaks in wards 45 and 46 (2012) and the MHC (2015).

Long-read sequencing demonstrated that the ST216 KPC-producing *E. coli* strain A1 isolate H124200646 (ward 46 [2012]) contained 2 plasmids, pKPC-CAD2 (307 kb; IncHI2/HI2A, with bla_{KPC} present) and pCAD3 (152 kb; IncFIB/FII, with bla_{KPC} absent); 83% of pKPC-CAD2 was highly similar (99% sequence identity) to pKPC-272 (282 kb, *Enterobacter cloacae*; GenBank accession no. CP008825.1), which was identified in a sink drain in the National Institutes of Health Clinical Centre in 2012 (16). The other long-read sequence, H151860951 (ward 4 [April 2015]), which was also an ST216 KPC-producing *E. coli* strain A1 isolate, contained a bla_{KPC} plasmid, pKPC-CAD1 (200 kb; IncFIB/FII), which had 99% sequence identity to pCAD3 over 76% of its length, together with a 48-kb contiguous region including bla_{KPC} that was 99% identical to part of pKPC-CAD2 (Fig. 4A). These results suggest the evolution of a bla_{KPC} plasmid similar to pKPC-272 in CMFT within ST216 KPC-producing *E. coli* strain A from 2012 to 2015, including recombination between pKPC-CAD2 and pCAD3, giving rise to pKPC-CAD1. Although plasmid typing based on mapping of short-read data to plasmid references should be interpreted cautiously, sequence comparisons with the outbreak plasmids pKPC-CAD1 and pKPC-CAD2 were consistent with the emergence of pKPC-CAD1 and its domination within ST216 KPC-producing *E. coli* strain A after 2014, as well as exchange of pKPC-CAD1, pKPC-CAD2, and pCAD3 with other *E. coli* STs (Fig. 3 and 4B).

Environmental CRE isolates. Thirty environmental CRE isolates from wards 3 and 4 were sequenced, 27 of which were isolated prior to the plumbing replacement and 16 of which were CR *E. coli*, as described above (13 isolated prior to the plumbing replacement). Eleven of the 16 *E. coli* isolates were ST216 KPC-producing *E. coli* strains (10 strain A1 and 1 strain A2), isolated on 8 separate days (in March, May, and September 2015 and February 2016), consistent with transmission between patients and the environment (Fig. 3) and persistence or reintroduction following the plumbing replacement. The other 14 isolates represented diverse KPC-producing CRE species, including *K. pneumoniae* ($n = 7$), *Citrobacter freundii* ($n = 4$), *Klebsiella oxytoca* ($n = 1$), *Enterobacter cloacae* ($n = 1$), and *Kluyvera intermedia* ($n = 1$). The KPC plasmids in these KPC-producing CRE isolates likely included the outbreak plasmids pKPC-CAD1 and pKPC-CAD2, pKpQIL, and other plasmids, consistent with the interspecies transfer of a diverse set of bla_{KPC} plasmids.

DISCUSSION

Our detailed analyses of the largest institutional KPC-producing *E. coli* outbreak described to date demonstrate a complex genetic and epidemiological picture, includ-
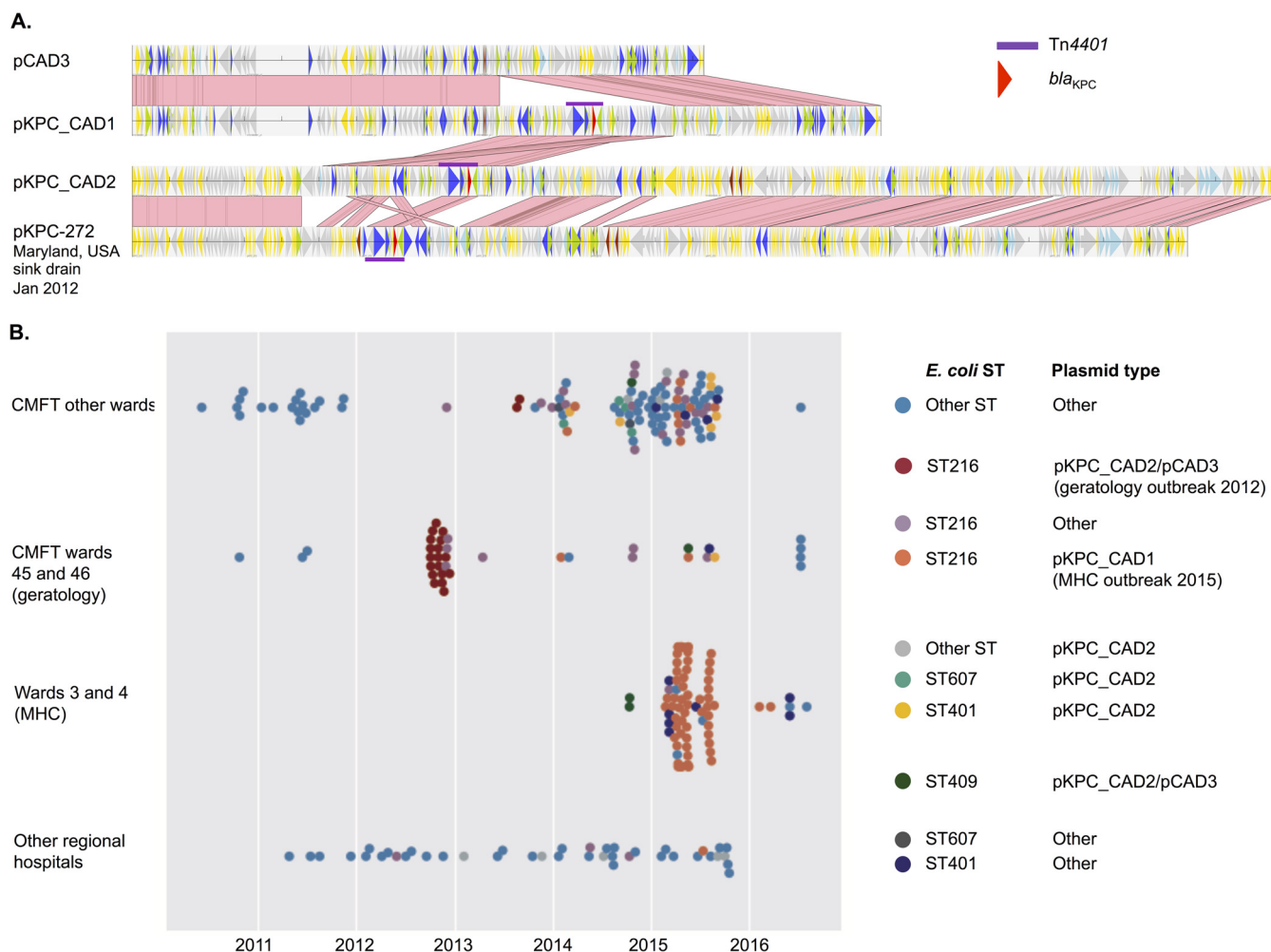
A.

B.

FIG 4 (A) Alignments of the 2012 MHC outbreak KPC plasmid pKPC-CAD2 (wards 45 and 46; Tn4401a plus bla_{KPC}) and the 2015 MHC KPC plasmid pKPC-CAD1 (Tn4401a plus bla_{KPC}), highlighting the recombination of the Tn4401a- and bla_{KPC}–harboring 48-kb segment from pKPC-CAD2 with pCAD3 to generate pKPC-CAD1. Regions of sequence homology are represented by pink links drawn between alignments. pKPC-272 (GenBank accession no. CP008825.1), a plasmid identified in an isolate from a sink drain at the National Institutes of Health Clinical Centre in Maryland in 2012, demonstrates significant sequence homology with pKPC-CAD2. (B) Incidence plot of different E. coli STs and likely MHC-related KPC plasmid types across hospital locations.

ing the emergence of ST216 KPC-producing E. coli strain A1 as a significant clone in CMFT, causing the major 2015 MHC outbreak, an antecedent outbreak in 2012, and sporadic cases and small clusters in other wards and regional health care settings. Plasmid-associated dissemination of bla_{KPC} to other E. coli lineages, including recognized high-risk clones such as ST131, was evident and the problem was substantial, with 514 confirmed patient acquisitions of CR E. coli over a 6-year period.

Environmental sampling on wards 3 and 4 confirmed that sinks and drains were colonized by multiple CRE strains, including the ST216 KPC-producing E. coli strains A1 and A2 and other CRE strains containing the outbreak KPC plasmids (pKPC-CAD1 and pKPC-CAD2), potentially representing a persistent reservoir between patient-associated outbreaks and plausibly explaining why this large outbreak was refractory to standard IPC bundles. Supporting this, the incidence of new CR E. coli detections declined substantially after ward plumbing replacement and temporary relocation of patients (Fig. 1A and 2A and C), consistent with a major contribution from the ward environment. After wards 3 and 4 reopened, however, the environment was rapidly recontaminated, including with ST216 KPC-producing E. coli strain A1, and CRE strains were again detected in patients, suggesting that this type of intervention has limited durability. National and international guidelines on CRE management recommend

rectal screening, strict contact precautions, isolation/cohorting of cases, and antimicrobial stewardship to limit transmission (12, 13, 17), all measures already being implemented in CMFT. Current guidelines do not address the control of large persistent outbreaks or provide advice on the sampling and management of environmental reservoirs, and there is limited evidence in support of any given measure (18). It is unclear why a particular strain of KPC-producing *E. coli* predominated in the outbreak described, as opposed to other CRE strains found contemporaneously in the environment; differences in the gastrointestinal colonization ability of species or an unidentified point source are potential hypotheses.

The response to this outbreak caused major disruption to the hospital and regional cardiac services. Given that almost all cases represented colonizations and not infections, the risks of associated delays in cardiac interventions were debated, although the impacts were not formally quantified. The estimated cost to CMFT of CRE in the first 8 months of 2015 was £5.2 million (19), and the MHC outbreak contributed significantly to this, with approximately £240,000 being spent on the ward 3/ward 4 plumbing replacement.

The study has several limitations, including its observational nature, with only 1 year of follow-up monitoring after the ward 3/ward 4 plumbing replacement. Limited environmental sampling might have meant that the extent of contamination and the diversity of CRE in environmental niches were underestimated. Environmental sampling was restricted to wards in which CRE outbreaks had been detected, and it focused predominantly on sink/drain sites (because initial sampling suggested that those sites were most heavily contaminated); however, component parts of each sink drainage system were not sampled consistently due to resource issues, and the relative prevalence of CRE isolation from any given site type needs to be interpreted with caution. We sequenced only single isolates cultured from individuals at any given time point, due to resource limitations, and therefore might have underestimated the CRE strain diversity within patients. Other non-*E. coli Enterobacteriaceae* strains were not comprehensively sequenced, possibly underestimating dissemination of pKPC-CAD1 and pKPC-CAD2; however, even our limited sequencing of CRE strains from the environment in 2015 identified those plasmids (and other KPC plasmids) in multiple species. Although genetic overlap between environmental and patient isolates was consistent with transmission between these compartments (Fig. 3), the numbers were too small to infer directionality. Of the predominant KPC plasmid types present within the ST216 KPC-producing *E. coli* strain A1 outbreak clone, one (pKPC_CAD2) was transferred to multiple *E. coli* STs (Fig. 3 and 4B), and another (pKPC_CAD1) might have contributed to the clone's success from 2014 (Fig. 4B), although the genetic and biological mechanisms underpinning this have not been explored.

Our experience highlights the limited evidence for managing large CRE outbreaks, including environmental sampling protocols and interventions, despite numerous centers reporting similar experiences with wastewater sites acting as CRE reservoirs (18, 20–23). Widespread colonization with KPC-producing *E. coli* is a concern, as *E. coli* is a common gastrointestinal colonizer and cause of infection, and any stable association between bla_KPC and *E. coli*, particularly in pathogenic lineages such as ST131 (Fig. 3), represents a significant clinical and transmission threat. Although our analyses focused on CRE, similar wider environmental contamination and dissemination of carbapenem-susceptible *Enterobacteriaceae* seem plausible. A more robust evidence base delineating transmission networks (including initial contamination of sink sites), drivers, and effective control measures (including differential impacts of decontamination methods on particular species and strains) is needed to minimize the financial, clinical, and social impacts of CRE outbreaks.

MATERIALS AND METHODS

Setting. CMFT is one of the largest hospital trusts in northwest England. The MHC manages >10,000 patients/year and in 2015 included two 28-bed inpatient wards (wards 3 and 4), an acute facility (ward 35), an intensive care unit, and a cardiac catheter laboratory. Ward 3 and ward 4 both included 3 bays and 4 single-patient side rooms, with a shared kitchen (see Fig. S1A and B in the supplemental material).

IPC measures. CRE screening and IPC measures, based on UK guidelines (11), were implemented Trust-wide from mid-2014. Enhanced measures were introduced in April 2015 in response to the MHC KPC-producing *E. coli* outbreak (Table S1). In addition, wards 3 and 4 (where most KPC-producing *E. coli* cases were observed) were closed to replace the plumbing infrastructure back to the drainage stacks (Fig. S2) from September 2015. Staff screening was not undertaken, consistent with national guidelines (11).

Patient CRE screening. Rectal swabs were screened for CRE using selective chromogenic agar, i.e., ChromID CARBA (bioMérieux) (published sensitivity, 89 to 100%; specificity, 95% [24–26]) to August 2014 and the Cepheid Xpert Carba-R assay (published sensitivity, 97 to 100%; specificity, 99% [27, 28]) from August 2014, along with an in-house multiplex PCR (bla_{KPC}, bla_{NDM}, and bla_{OXA-48}) from November 2014. The Cepheid assay was used for specimens from patients with admissions to the Trust in the past 12 months, those admitted from overseas, or those due to be transferred to a district general hospital (to facilitate transfer planning). All other samples were tested using the multiplex PCR. Species identification of isolates was performed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker).

Epidemiological analyses. CMFT electronic bacteriology records were linked, based on NHS numbers, to patient administration data (1 January 2010 to 1 January 2017) and anonymized, and the first CRE-positive test result per patient (rectal screening or clinical specimen) was considered in the evaluation of CRE incidence trends. Trends and the impact of IPC interventions were analyzed retrospectively.

Because CMFT CRE screening rates changed over time in response to national guidance and local IPC interventions and a key aim was to evaluate specifically the impact of ward closure and a radical plumbing intervention in the MHC on CRE acquisition rates, we considered CRE detection rates in 4 periods delineated by 3 time points, namely, the implementation of national carbapenemase-producing *Enterobacteriaceae* (CPE) IPC policy in mid-2014 (which substantially increased the number of screens performed), the beginning of the MHC-specific intervention (patient relocation and plumbing infrastructure replacement in wards 3 and 4), and the end of the MHC intervention.

First CRE-positive screens were used as a pragmatic proxy for CRE acquisition (i.e., a "case"), given that 89% of patients with first CRE-positive results in the MHC had a negative rectal screen within the preceding 14 days (79% within 7 days) (Fig. S3 to S5). Information on specific carbapenemase mechanisms was not consistently available for all isolates, hampering our ability to perform these analyses specifically by carbapenemase gene family (Table S2).

We tested the hypothesis that CRE acquisitions (reflected by first CRE-positive screens) changed in the MHC more than in other hospital wards following the ward 3/ward 4 closure and plumbing intervention, using negative binomial regression models for the weekly counts of first (per person) CRE detection ≥2 days postadmission (i.e. cases), using weekly numbers of persons screened ≥2 days postadmission as an offset (i.e., adjusting for screening rates), and counting each patient as screened as long as they had ≥1 screen per week. Models were fitted (R v3.4.1) for CRE, CR *E. coli*, and CR *K. pneumoniae*. We included period and ward location (MHC versus other wards) as independent variables, with interaction terms for period and location (see the supplemental material for details).

Environmental sampling and sample processing. In 2015, environmental samples were taken from ward sites using charcoal swabs and were cultured on ChromID CARBA for 18 h at 37°C. After January 2016, ~20 ml of wastewater was aspirated from sink P-traps, shower drains, or toilets. Aspirates were centrifuged at 4,000 rpm for 10 min, 15 ml of supernatant was discarded, and the pellet was resuspended in the remaining 5 ml. One milliliter of sample was then incubated aerobically overnight at ~37°C in 5 ml of trypticase soy broth with an ertapenem disc; the multiplex PCR (as above) was performed on broths to identify bla_{KPC}-positive samples for subsequent culture on ChromID CARBA. Environmental sampling prior to January 2016 was not systematic; after January 2016, 75 wastewater sites on wards 3 and 4 were sampled fortnightly on rotation (one half of the sites 1 week and the other half the next); these sites included toilets, sink basins, and sink drains.

Genome sequencing and sequence data analysis. To provide genetic context for the outbreak, we sequenced retrievable, archived, KPC-producing *E. coli* patient and environmental isolates from CMFT and patient isolates collected for regional public health surveillance (see the supplementary methods and Table S3 in the supplemental material). We also sequenced a small subset of non-*E. coli* environmental CRE isolates that had been stored ($n = 14$) *ad hoc* as part of outbreak sampling prior to the plumbing replacement.

For Illumina sequencing (HiSeq 2500; 150-bp PE reads), DNA was extracted using the QuickGene system (Fujifilm, Japan), with an additional mechanical lysis step following chemical lysis (FastPrep; MP Biomedicals, USA). Two outbreak isolates (H124200646 and H151860951) were selected for long-read sequencing based on Illumina data. For long-read sequencing (with a PacBio [$n = 1$] or MinION [$n = 1$] system), DNA was extracted using the Qiagen genomic tip 100/G kit (Qiagen, Netherlands) (see the supplementary methods).

In silico species identification was performed using Kraken (29). Illumina reads were then mapped to species-specific references (*E. coli* CFT073 [GenBank accession no. AE014075.1] and the ST216 reference H151860951), and base-calling was performed as described previously (30). *De novo* assembly was performed using SPAdes v3.6 (31), and resistance gene, bla_{KPC} plasmid, and Tn*4401* typing was performed using BLASTn and mapping-based approaches (see the supplementary methods and Table S3).

Two-dimensional reads were extracted from MinION sequence data using poretools (32); hybrid-SPAdes (31) and Canu (33) were used to generate *de novo* hybrid assemblies from MinION and Illumina data (see the supplementary methods in the supplemental material). PacBio sequence data were *de novo*

assembled using HGAP3 (34). *E. coli* phylogenies were reconstructed using IQ-Tree (35) and Clonal-FrameML (36) and were visualized in iTOL (37) (see the supplementary methods).

Ethics approval. Because the investigations formed part of a Trust board-approved outbreak response, ethics approval was not required under NHS governance arrangements (see the supplementary methods).

Accession number(s). Sequencing data are available under NCBI BioProject PRJNA379782.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC .01689-18.

SUPPLEMENTAL FILE 1, PDF file, 1 MB.

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