A large, refractory nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-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) are a health threat, but effective control interventions remain unclear. Hospital wastewater sites are increasingly highlighted as important potential reservoirs. We investigated a large *Klebsiella pneumoniae* carbapenemase (KPC)-producing *E. coli* (KPC-EC) outbreak and wider CRE incidence trends over eight years in the Central Manchester Foundation NHS Trust (CMFT), UK, to determine the impact of Infection Prevention and Control measures.

Bacteriology and patient administration data (2009-2017) were linked; a subset of CMFT/regional KPC-EC isolates (n=268) was sequenced. Control interventions followed international guidelines and included cohorting, rectal screening (n=184,539 screens), environmental sampling, enhanced cleaning, and ward closure/plumbing replacement. Segmented regression of time trends of CRE detections was used to evaluate the impact of interventions on CRE incidence.

Genomic analysis (n=268 isolates) identified spread of a KPC-EC outbreak clone (ST216, strain-A; n=125) amongst patients and the environment, particularly on two cardiac wards (W3/W4), despite control measures. ST216 strain-A had caused an antecedent outbreak, and shared its KPC plasmids with other *E. coli* lineages and *Enterobacteriaceae*. CRE acquisition incidence declined after W3/W4 closure and plumbing replacement, suggesting an environmental contribution. However, W3/W4 wastewater sites were rapidly re-colonised with CRE and patient CRE acquisitions recurred, albeit at lower rates.

Patient relocation and plumbing replacement were associated with control of a clonal KPC-EC outbreak; however, environmental contamination with CRE and patient CRE acquisitions

recurred rapidly following this intervention. The large numbers of cases and persistence of *bla*$_{KPC}$ in *E. coli*, including pathogenic lineages, is a concern.

INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a global public health threat(1). Major carbapenemases include the metallo-beta-lactamases, some oxacillinases and the *Klebsiella pneumoniae* carbapenemase (KPC, encoded by bla_{KPC}), one of the commonest carbapenemases globally(2). Transfer of carbapenemase genes on mobile genetic elements has resulted in the rapid, inter-species 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 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-*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 is of concern.

CRE detections in England have increased since 2008(9), and are approximately ten times the national average in Greater Manchester(10). Central Manchester University Hospitals NHS Foundation Trust (CMFT) has experienced an on-going multi-species 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 faecal colonisation with KPC-producing *E. coli* (KPC-EC) was detected in the Manchester Heart Centre (MHC) at the Manchester Royal Infirmary (MRI; part of CMFT). We retrospectively investigated the genomic epidemiology and evidence for nosocomial transmission of KPC-EC and KPC plasmids isolated from patients

and the environment in this context, and assessed the impact of guideline-compliant IPC bundles on CRE and KPC-EC incidence.

RESULTS

High prevalence of CRE colonisation in the MHC

Between 01/Apr-30/Dec/2014, 23 new CRE-colonised individuals were detected on the MHC, including two with *E. coli* (Fig.1A). A CRE outbreak was declared on 02/Jan/2015 when six new CRE-colonised individuals were identified (four with bla_{KPC}, two with bla_{NDM}; no *E. coli*). Consequently, intensified IPC measures were implemented (Table S1; Fig.1B), and W3/W4 were closed (06/Jan/2015), terminally cleaned (hypochlorite), and decontaminated (hydrogen peroxide vapour). W3 was re-opened on 11/Jan/2015 and W4 on 23/Jan/2015; high-risk patients (CRE previously detected/history of hospitalisation abroad or in UK hospital with known CRE transmission in 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 Trust-wide CRE screening program (>110 screens/day; Table S2). Between 01/Sep/2014-30/Dec/2014, screening transitioned from culture- to PCR-based methods: during this period 16,612 samples from 7,239 inpatients were screened using either culture (n=9,808), or PCR+culture (n=6,804), with an overall CRE prevalence of 3.8% (438 positive samples, 272 patients). Molecular mechanism data for 135/163 (83%) PCR-positives indicated bla_{KPC} accounted for most carbapenem resistance (97%).

KPC-*E. coli* outbreak despite IPC interventions

Following the implementation of enhanced IPC activity, there was a further sharp increase in the number of CRE-colonised patients detected from 09/Mar/2015 (Fig.1A; CR-*E. coli* and other species, mostly bla_{KPC}, a few bla_{NDM}). W3 was again closed to admissions (11/Mar/2015-28/Mar/2015) and environmental decontamination repeated; the following week W4 was closed after detection of additional CRE-colonised patients (Figs.1A, 1B). From 01/April/2015 KPC-EC predominated in the outbreak (Fig. 1A).

From April-September 2015, W3/W4 were closed repeatedly, with two peaks in KPC-EC patient colonisation (April-May and August; Fig.1B). W3 capacity was reduced to 10 day-case beds (12/Aug/2015; day-case patients not screened for CRE) and W4 to 12 in-patient beds. Between 10/Aug/2015-28/Sep/2015, there were 27 new KPC-EC colonisations detected on the MHC (Fig.1A), and two cases with other KPC-*Enterobacteriaceae*. Of 88 KPC-EC cases between 24/Feb/2015-28/Sep 2015, 86 (98%) represented colonisations only; one individual additionally had a UTI and one a sternal wound infection (treated with gentamicin and ciprofloxacin respectively, to which the isolates were susceptible).

Carbapenem-resistant *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 positives by patient from clinical/screening isolates, 2010-2016 inclusive), and including a separate outbreak on the geratology wards (W45/46) in late 2012 (Figs.2A,2B). Of these, 434 cases were detected on ≥day 2 of admission, and a further 80 on day 0-1 of admission. Case peaks were not related to screening policy changes/rates (Fig.S6). CR-*E. coli* were almost invariably detected from rectal screening (420/434 cases, 97%).

Environmental sampling yielded CRE from sinks/drains

Intermittent environmental sampling was undertaken to identify potential reservoirs. Overall, 927 samples from 833 sites were taken 09/Apr-17/Nov/2015; 355 (38%) samples from 333 (40%) sites were from W3/W4, and the remainder from eleven other wards. 850 samples were from sink/drain/shower/bath sites, 18 from toilets/hoppers/sluices, and 33 from high-touch sites (including keyboards, door handles, sponges etc.; labelling unclear for 26 samples). Eighty-five samples (9%) and 72 sites (9%) were CRE-positive (26/355 samples [7%], 21/333 sites [6%] on W3/4). 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); other (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.

Of ten sites yielding 11 KPC-EC isolates, five were in the W3/W4 kitchen (14-18/May/2015 [n=4], 10/Sep/2015 [n=1]), one a W4 staff sink (14/May/2015), and four from kitchen sinks/drains on wards 31/32 (sampling in response to a separate ward 31/32 outbreak, 12-17/Nov/2015). W3/W4 sink-specific interventions included sink trap replacement for CRE-colonised sinks (16/Apr/2015, 31/Jul/2015, 11/Aug/2015) and horizontal pipework cleaning with a brush to try and remove biofilm (11/Aug/2015).

Cardiac service relocation and decline in CRE colonisation incidence

Given the on-going difficulty in preventing KPC-EC acquisitions, and the isolation of KPC-EC from sinks/drain sites, W3/W4 were closed from 25/Sep/2015 and patients re-located to another ward to allow replacement of the plumbing infrastructure back to the central drainage stacks. Replaceable sink plughole devices designed to prevent water aerosolisation in the sink U-bend and limit biofilm formation were installed (HygieneSiphon, Aquafree;

https://www.aqua-free.com/en/gb/medical-water-hygiene/products/medical-application/produkt/Ressort/product/hygienesiphon/).

Controlling for screening and compared to the period immediately pre-intervention (when screening policy was the same), the incidence of first detection of any CRE or CR-*E. coli* fell significantly following the plumbing intervention, both in the MHC and elsewhere in the hospital (Fig.2C, Table 1); but the decline in incidence was significantly greater in the MHC ($p_{heterogeneity}<0.001$), where incidence fell by 89% for any CRE and by 98% for CR-*E. coli*. Incidence of CR-*K. pneumoniae* also fell significantly in both settings, but there was no evidence that the decline differed between the two settings ($p_{heterogeneity}=0.31$, Table 1). However, when patients were transferred back to W3/W4 (from 18/Jan/2016), CR-*E. coli* continued to be detected in patients (six first detections in 2016, Fig.2A). Patient colonisation with other CRE was also observed, in similar numbers to 2014 (Fig.1A); environmental contamination with CRE in sink/wastewater sites recurred rapidly (Fig.1A), and two environmental sites (both ward utility room sink drains) were CRE-positive even prior to patient re-admissions to the ward, suggesting residual contamination after the plumbing replacement, or re-introduction following the plumbing replacement but prior to patient readmissions.

Genomic epidemiology of KPC-*EC*

268 clinical and environmental CR-*E. coli* isolates were sequenced. These included 82 isolates from the MHC (2015-2016 [16 environmental]), 36 from W45/W46 (2010-2016), 109 from other CMFT wards/units, and 41 from other regional hospitals (Table S3). Nine isolates were bla_{KPC}-negative on sequencing; five of these contained bla_{OXA-48}, one $bla_{OXA-181}$, and one bla_{NDM-5}, with no known carbapenem resistance mechanisms identified in the

remaining two. The 259 KPC-EC isolates included all 16 environmental CR-*E. coli*, 158 isolates which were the first CR-*E. coli* cultured from patients, 38 sequentially cultured CR-*E. coli* from patients (longitudinal cultures from 12 patients). For 47/259 isolates sequencing and patient epidemiological identifiers could not be linked.

Forty sequence types (STs), including known pathogenic lineages (e.g. ST131), occurred amongst the KPC-EC isolates (Fig.3, Table S3), highlighting regional KPC-EC diversity. In contrast, 67/80 (84%) MHC isolates were ST216 versus 59/179 (33%) elsewhere. ST216 has rarely been reported in other settings.

ST216 KPC-EC

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

All but three ST216 isolates carried bla_{KPC-2} in a Tn*4401*a transposon(14), typically associated with high-level bla_{KPC} expression(15), and flanked by a 5-bp target site duplication, AGTTG, previously only observed with the Tn*4401*b isoform in an isolate from Colombia (Fig.3, 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-EC outbreak strains (Figs.3, S8). Overall, these results demonstrated clonal expansion of specific KPC-EC strains, with significant accessory genome mobility. Most notable was the emergence and persistence of ST216 KPC-EC strain-A1, isolated from patients and the environment over four years, and causing outbreaks on W45/W46 (2012) and the MHC (2015).

Long-read sequencing demonstrated that the ST216 KPC-EC strain-A1 isolate H124200646 (W46, 2012) contained two plasmids, pKPC-CAD2 (307kb; IncHI2/HI2A; bla_{KPC} present) and pCAD3 (152kb; IncFIB/FII; bla_{KPC} absent). 83% of pKPC-CAD2 was highly similar (99% sequence identity) to pKPC-272 (282kb, *E. cloacae*, GenBank accession CP008825.1), identified in a sink drain at the National Institutes of Health Clinical Centre, Maryland, USA, 2012(16). In contrast, the other long-read sequence, H151860951 (W4, April 2015), also an ST216 KPC-EC strain-A1 isolate, contained a bla_{KPC}-plasmid pKPC-CAD1 (200kb; IncFIB/FII), which had 99% sequence identity over 76% of its length to pCAD3, together with a 48kb 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 an ST216 KPC-EC strain-A from 2012-2015, including recombination between pKPC-CAD2 and pCAD3 giving rise to pKPC-CAD1.

Although plasmid typing based on mapping 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-EC strain-A post-2014; and exchange of pKPC-CAD1/pKPC-CAD2/pCAD3 with other *E. coli* STs (Fig.3; Fig.4B).

Environmental CRE isolates

Thirty environmental carbapenem-resistant *Enterobacteriaceae* isolates from W3/W4 were sequenced, 27 isolated prior to the plumbing replacement, and 16 of which were CR-*E. coli* , as described above (13 prior to plumbing replacement). 11/16 *E. coli* were ST216 KPC-EC (ten strain-A1, one strain-A2), isolated on eight separate days (in March, May, September 2015, February 2016), and consistent with transmission between patients and the environment (Fig.3), and persistence/reintroduction following plumbing replacement. The other 14 isolates represented diverse KPC-CRE, 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-CRE likely included the outbreak plasmids pKPC-CAD1 and pKPC-CAD2, pKpQIL, and others, consistent with the inter-species transfer of a diverse set of bla_{KPC} plasmids.

DISCUSSION

Our detailed analyses of the largest institutional KPC-*E. coli* outbreak described to date demonstrate a complex genetic and epidemiological picture including the emergence of ST216 KPC-EC strain-A1 as a significant clone in CMFT, causing the major 2015 MHC outbreak, an antecedent outbreak in 2012, and sporadic cases/small clusters in other wards and regional healthcare settings. Plasmid-associated dissemination of bla_{KPC} to other *E. coli* lineages, including recognised “high-risk” clones such as ST131, was evident, and the problem substantial, with 514 confirmed patient acquisitions of CR-*E. coli* over a six-year period.

Environmental sampling on W3/W4 confirmed that sinks/drains were colonised by multiple CRE, including the ST216 KPC-EC strains-A1/A2 and other CRE containing the outbreak KPC plasmids (pKPC-CAD1, 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 (Figs.1A, 2A, 2C), consistent with a major contribution from the ward environment. However, after W3/W4 reopened the environment was rapidly re-contaminated, including with ST216 KPC-EC strain-A1, and CRE 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 implemented in CMFT. Current guidelines do not address the control of large, persistent outbreaks, or advise 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-*E. coli* predominated in the outbreak described, as opposed to other CRE contemporaneously found in the environment - differences in gastrointestinal colonisation ability of species, or an unidentified point source could be potential hypotheses.

The response to this outbreak caused major disruption to the hospital and regional cardiac services. Given that almost all cases represented colonisations and not infections, the risks of associated delays in cardiac interventions were debated, although the impact of these were not formally quantified. The estimated cost of CRE to CMFT in the first 8 months of 2015 was £5.2m(19), and the MHC outbreak contributed significantly to this, with ~£240,000 spent on the W3/W4 plumbing replacement.

The study has several limitations, including its observational nature, with only a year of follow-up after the W3/W4 plumbing replacement. Limited environmental sampling may have meant that the extent of contamination and diversity of CRE in environmental niches was underestimated. Environmental sampling was restricted to wards on which CRE outbreaks were detected and focused predominantly on sink/drain sites (as initial sampling suggested these were most heavily contaminated); however, component parts of each sink drainage system were not sampled consistently due to resource issues and so relative CRE isolation prevalence from any given site type needs to be interpreted with caution. We only sequenced single isolates cultured from individuals at any given time-point due to resource limitations, and may therefore have underestimated the CRE strain diversity within patients. Other non-*E. coli Enterobacteriaceae* were not comprehensively sequenced, possibly underestimating dissemination of pKPC-CAD1 and pKPC-CAD2; however, even our limited sequencing of CREs from the environment in 2015 identified these 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 two predominant KPC plasmid types present within the ST216 KPC-EC strain-A1 outbreak clone, one (pKPC_CAD2) was transferred to multiple *E. coli* STs (Figs.3, 4B), and another (pKPC_CAD1) may have contributed to the clone's success from 2014 (Fig.4B), although the genetic/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 centres reporting similar experiences with wastewater sites acting as CRE reservoirs(18, 20-23). Widespread

colonisation with KPC-EC 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/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-bedded inpatient wards (Wards 3 [W3] and 4 [W4]), an acute facility (Ward 35), intensive care unit, and cardiac catheter laboratory. Both W3 and W4 comprised three bays, four single-patient side-rooms, and a shared kitchen (Figs. S1A, S1B).

IPC measures

CRE screening/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-EC outbreak (Table S1). In addition, W3/W4 (where most KPC-EC cases were observed) were closed to replace 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 (ChromID CARBA, Biomerieux; published sensitivity: 89-100%, specificity: 95%(24-26)) to August 2014, and the Cepheid Xpert Carba-R assay (published sensitivity: 97-100%, specificity: 99%(27, 28)) from August 2014, alongside an in-house multiplex PCR (bla_{KPC}, bla_{NDM}, bla_{OXA-48}) from November 2014. The Cepheid assay was used on specimens from patients with admissions to the Trust in the last 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 MALDI-TOF mass spectrometry (Bruker).

Epidemiological analyses

CMFT electronic bacteriology records were linked on NHS number to patient administration data (01/Jan/2010-01/Jan/2017) and anonymised, 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 analysed retrospectively.

As CMFT CRE screening rates changed over time in response to national guidance and local IPC interventions, and a key aim was to specifically evaluate the impact of ward closure and a radical plumbing intervention in the MHC on CRE acquisition rates, we considered CRE detection rates in four periods delineated by three time points: the implementation of national 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 on W3/W4), 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 first-CRE positive on the MHC had a negative rectal screen within the preceding 14 days (79% within 7 days; Figs S3-5). Information on specific carbapenemase mechanism was not consistently available for all isolates, precluding 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 on the MHC more than other hospital wards following the W3/W4 closure/plumbing intervention using negative binomial regression models for the weekly counts of first (per person) CRE detection ≥2 days post-admission (i.e. cases), using weekly numbers of persons screened ≥2 days post-admission as an offset (i.e. adjusting for screening rates, and counting each patient as screened as long as they had one or more screens per week). Models were fitted (R v3.4.1) for CRE, carbapenem-resistant *E. coli* (CR-*E. coli*), and carbapenem-resistant *K. pneumoniae* (CR-*K. pneumoniae*). We included period and ward location (MHC versus other wards) as independent variables, plus interaction terms between period and location (details in Supplementary Methods).

Environmental sampling and sample processing

In 2015, environmental samples were taken from ward sites using charcoal swabs, and cultured on ChromID CARBA (18 hours, 37°C). After January 2016, ~20mls of wastewater was aspirated from sink P-traps, shower drains or toilets. Aspirates were centrifuged at 4000rpm for 10mins, 15mls of supernatant were discarded, and the pellet was re-suspended in the remaining 5mls. One ml of sample was then incubated aerobically overnight (~37°C) in 5mls 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 W3/W4 were sampled fortnightly on rotation (half of the sites one week and 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-EC patient and environmental isolates from CMFT, and patient isolates collected for regional public health surveillance (Supplementary Methods; Table S3). We also sequenced a small subset of non-*E. coli* environmental CRE that had been stored (n=14) ad hoc as part of outbreak sampling prior to the plumbing replacement.

For Illumina sequencing (HiSeq 2500, 150bp PE reads), DNA was extracted using Quickgene (Fujifilm, Japan), with an additional mechanical lysis step following chemical lysis (FastPrep, MP Biomedicals, USA). Two outbreak isolates (H124200646, H151860951) were selected for long-read sequencing based on Illumina data. For long-read sequencing (PacBio [n=1], MinION [n=1]) DNA was extracted using the Qiagen Genomic tip 100/G kit (Qiagen, Netherlands) (Supplementary Methods; sequencing data available under NCBI BioProject PRJNA379782).

In silico species identification was performed using Kraken(29). Illumina reads were then mapped to species-specific references (*E. coli* CFT073 [AE014075.1], and the ST216 reference H151860951) and base-calling performed as previously(30). *De novo* assembly was performed using SPAdes (v3.6)(31) and resistance gene, *bla*$_{KPC}$ plasmid and Tn*4401* typing using BLASTn and mapping-based approaches (Supplementary Methods; Table S3).

2D-reads were extracted from MinION sequence data using poretools(32); hybridSPAdes(31) and Canu(33) were used to generate *de novo* hybrid assemblies from MinION+Illumina data (Supplementary Methods). PacBio sequence data were *de novo* assembled using HGAP3(34). *E. coli* phylogenies were reconstructed using IQTree(35) and ClonalFrameML(36), and visualised in iTOL(37) (Supplementary Methods).

Ethical approval

As the investigations formed part of a Trust board-approved outbreak response, ethical approval was not required under NHS governance arrangements (Supplementary Methods).

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605

606 **FIGURE LEGENDS**

607 **Figure 1.A.** The number of individuals on the Manchester Heart Centre (MHC) wards with
608 first-ever positive carbapenem-resistant *Enterobacteriaceae* detection, by week, stratified by
609 genus group/species of the organism isolated. *bla*$_{KPC}$-positive *Enterobacteriaceae* detected in
610 environmental samples over the same timeframe are also shown. The MHC outbreak was
611 declared by the Infection Prevention and Control Team in the first week in 2015 (arrow). **B.**
612 Timeline of infection prevention and control measures instituted. **C.** Bed occupancy per week
613 in the MHC, demonstrating the impact of infection control interventions on clinical activity.
614
615 **Figure 2.A, B.** Counts of individuals with first carbapenem-resistant *E. coli* detection by
616 ward location. Detections on days 0 and 1 of admission are excluded. Faint vertical lines
617 correspond to the boundaries of four time periods: P1-prior to implementation of systematic
618 carbapenemase-producing *Enterobacteriaceae* (CPE) rectal screening policy; P2-
619 implementation of CPE rectal screening policy consistent with national guidance; P3-closure
620 of W3/W4 and replacement of plumbing infrastructure; P4-reopening of W3/W4 to patient
621 admissions. **C.** Panels show incidence rate ratios for rates of first positive carbapenem-
622 resistant *E. coli* detection, carbapenem-resistant *K. pneumoniae* detection, and any
623 carbapenem-resistant *Enterobacteriaceae* detection ≥2 days post-admission relative to period
624 P2 in the same location (Manchester Heart Centre [MHC] vs rest of CMFT). An IRR is not
625 shown for P3 in the MHC due to unit closure during this time period to facilitate plumbing
626 replacement.
627
628 **Figure 3**. Recombination-corrected phylogeny of 259 sequenced KPC-*E. coli* (and nine *E.*
629 *coli* isolates that were *bla*$_{KPC}$ negative on sequencing) from CMFT and other regional
630 hospitals in northwest England, annotated with collection date, ward/centre location, Tn*4401*

type and outbreak plasmid types. 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 this. KPC-EC isolates from a Public Health England (PHE) project sequencing the first ten KPC-*Enterobacteriaceae* from hospitals in northwest England (2009-2014) are denoted "regional study isolates". "Environmental isolates" denote KPC-EC cultured during an initial environmental prevalence survey on W3/W4 (10/Mar/2015); any KPC-EC isolated as part of subsequent, intermittent IPC-associated environmental sampling (09/Apr/2015-17/Nov/15); and isolates available at the time of analysis from environmental and patient samples from a separate, on-going study (commenced January 2016).

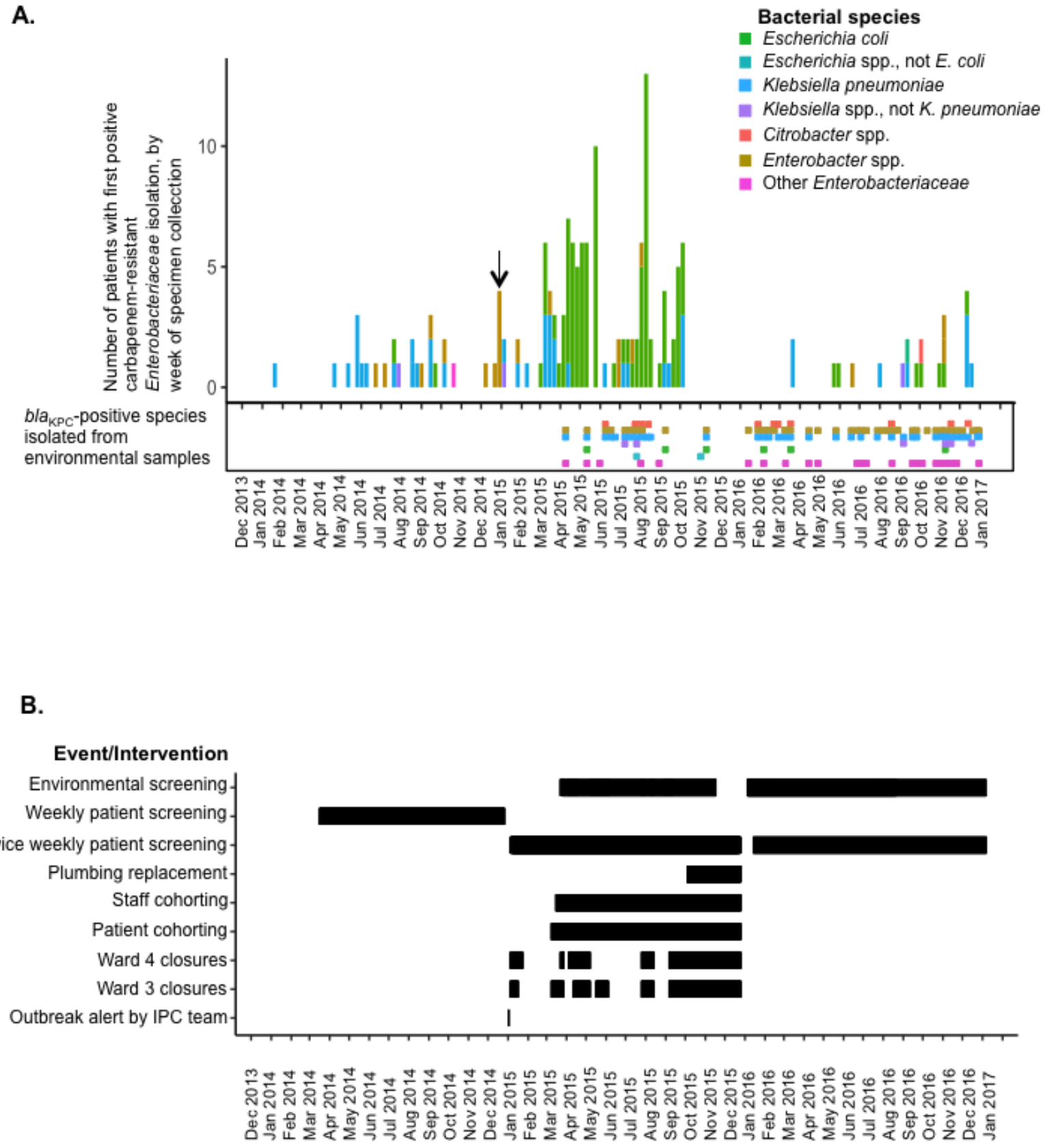
Figure 4.A. Alignments of Manchester Heart Centre (MHC) outbreak 2012 KPC plasmid pKPC-CAD2 (W45/46; Tn*4401a*+bla_{KPC}) and the 2015 MHC KPC plasmid pKPC-CAD1 (Tn*4401a*+bla_{KPC}), highlighting the recombination of the Tn*4401a*+bla_{KPC}-harbouring 48kb segment from pKPC-CAD2 with pCAD3 to generate pKPC-CAD1. Regions of sequence homology are represented by salmon-pink links drawn between alignments. pKPC-272 (GenBank accession CP008825.1), a plasmid identified in an isolate in a sink drain at the National Institutes of Health Clinical Centre, Maryland, USA, 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.

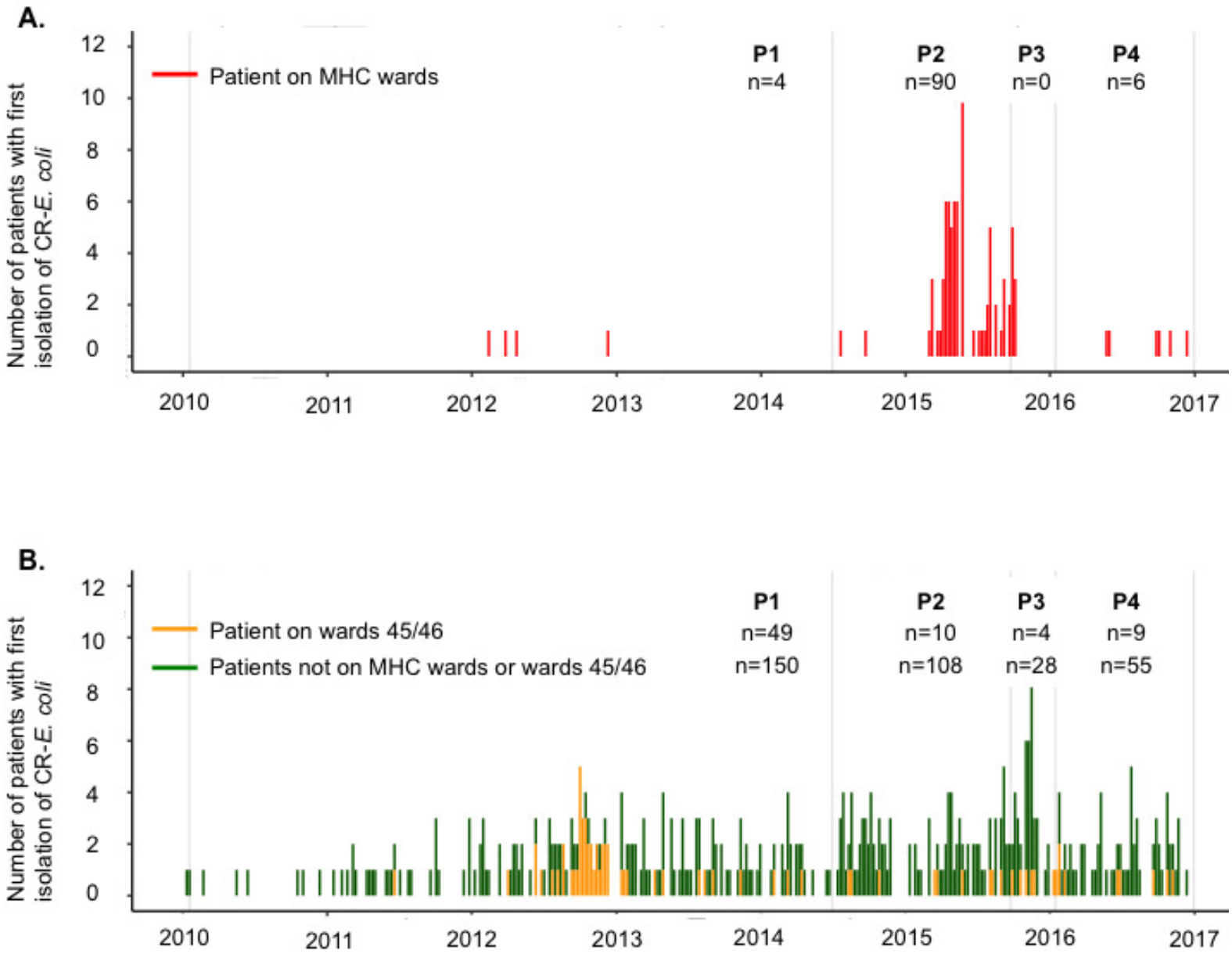
652 **Table 1. Incidence rate ratios (IRR) for detection from screening swabs 2 or more days after admission, a proxy marker of acquisition,**
653 **in Central Manchester Foundation NHS Trust of: (i) all carbapenem-resistant *Enterobacteriaceae*; (ii) carbapenem-resistant *E. coli*; and**
654 **(iii) carbapenem-resistant *K. pneumoniae*, modelling the impact of the W3/W4 closures and plumbing replacement on acquisition.** Four
655 time periods were evaluated: P1-prior to implementation of systematic carbapenemase-producing *Enterobacteriaceae* (CPE) rectal screening
656 policy; P2-implementation of CPE rectal screening policy consistent with national guidance; P3-closure of W3/W4 and replacement of plumbing
657 infrastructure; P4-reopening of W3/W4 to patient admissions.

	All carbapenem-resistant *Enterobacteriaceae* (number of cases=3,086)			**Carbapenem-resistant *E. coli* (number of cases=502)**			**Carbapenem-resistant *K. pneumoniae* (number of cases=1,134)**		
	IRR	**95% CI**	**P**	**IRR**	**95% CI**	**P**	**IRR**	**95% CI**	**p**
Manchester Heart Centre (MHC)									
Week 03 2010 to week 26 2014 (P1)	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 (P2; reference period*)	1.00			1.00			1.00		
Week 40 2015 to week 02 2016 (P3; W3/W4 closed)	-	-	-	-	-	-	-	-	-

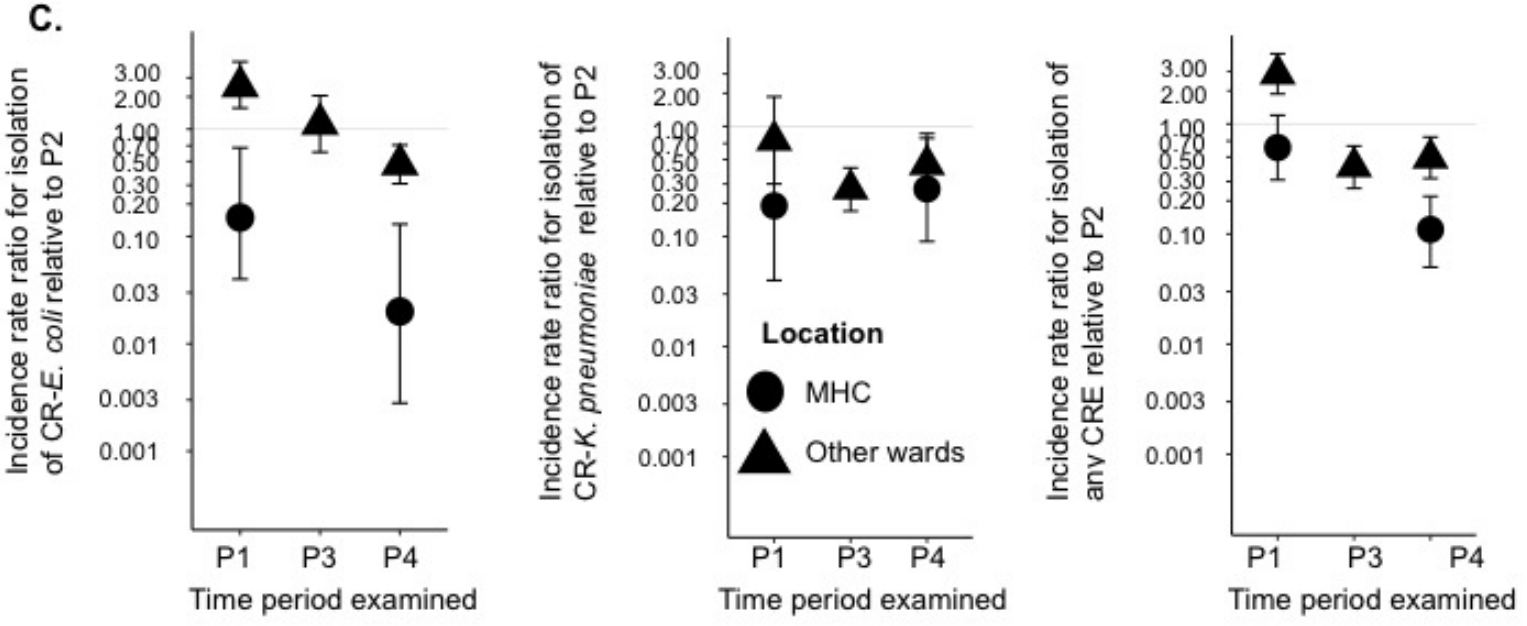
Week 03 2016 to week 52 2016 (P4)	0.11	0.05-0.22	<0.001	0.02	0.00-0.14	<0.001	0.27	0.09-0.78	0.015
Other hospital locations									
Week 03 2010 to week 26 2014 (P1)	2.85	1.87-4.34	<0.001	2.51	1.57-4.03	<0.001	0.75	0.30-1.86	0.53
Week 27 2014 to week 39 2015 (P2; reference period)	1.00			1.00			1.00		
Week 40 2015 to week 02 2016 (P3)	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 03 2016 to week 52 2016 (P4)	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 (P2)	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 between reduction in MHC vs other location									
Week 03 2010 to week 26 2014 (P1)			<0.001			0.001			0.098
Week 40 2015 to week 02 2016 (P3)			-			-			-
Week 03 2016 to week 52 2016 (P4)			<0.001			0.003			0.31

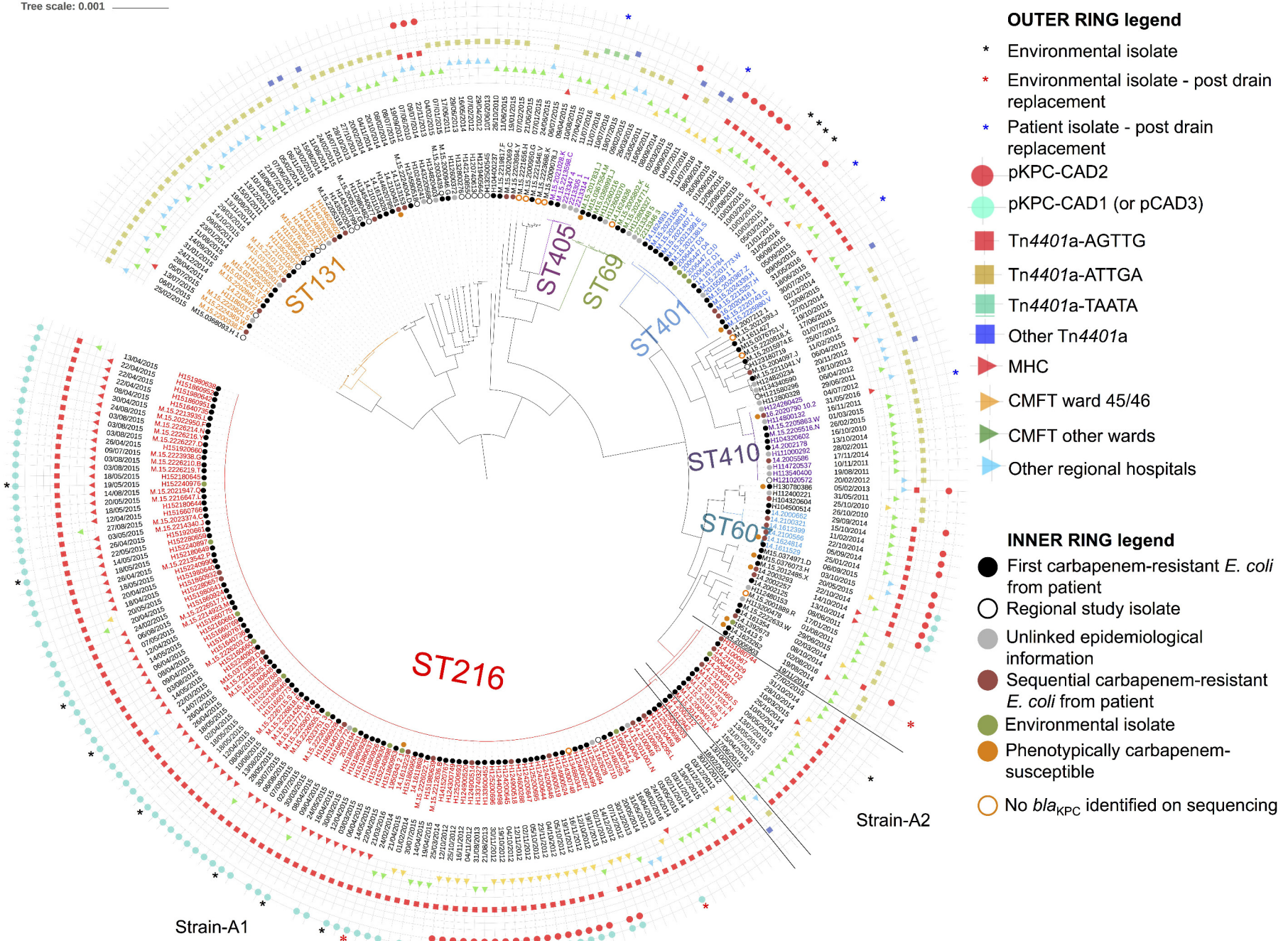
* P2 chosen as reference period because of change in screening policy between P1 and P2 (Table S2, Fig.S6), meaning that a greater incidence would be expected in P2 due to more patients being screened every week.

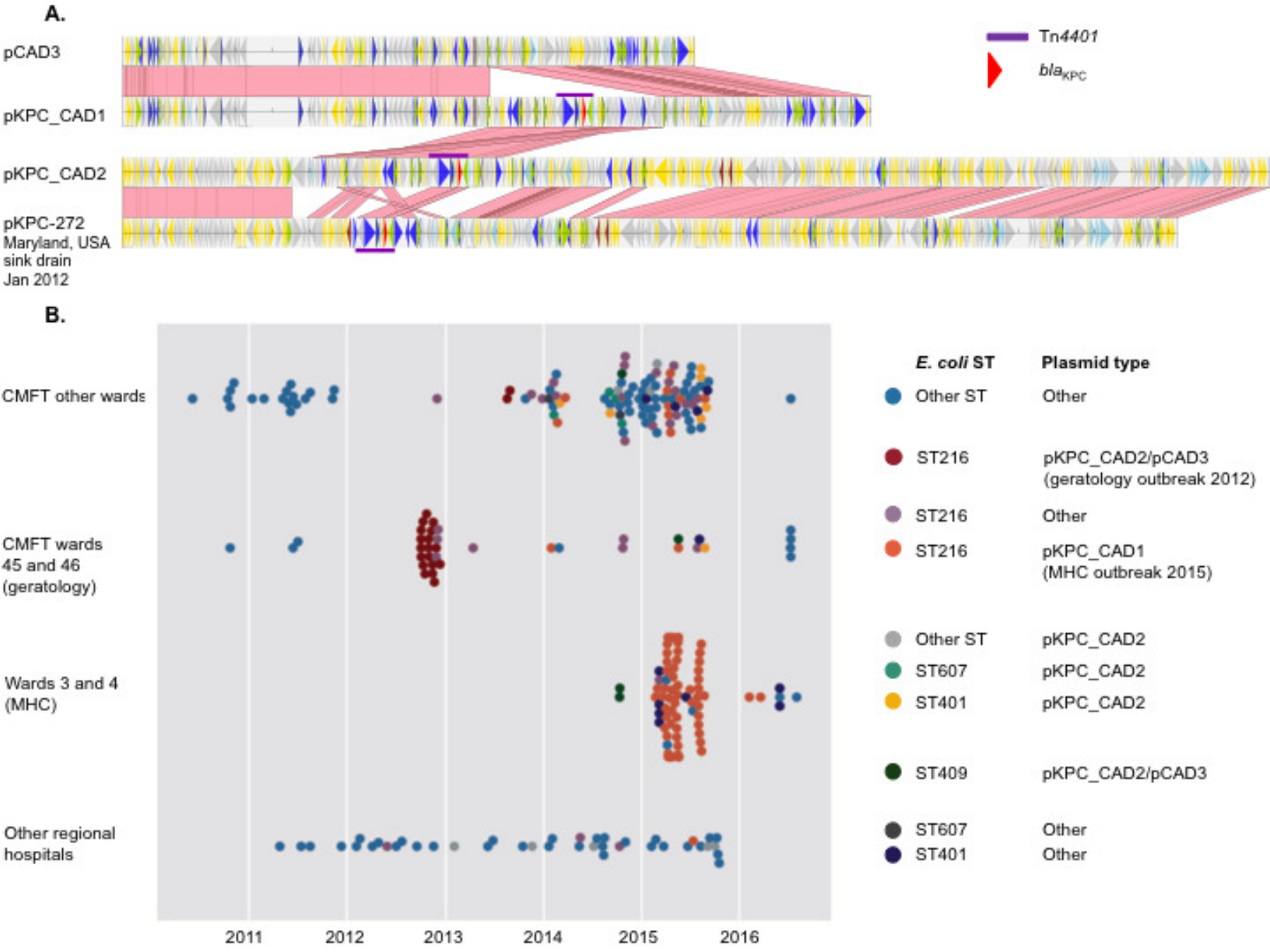

A.
Bacterial species
Escherichia coli
Escherichia spp., not E. coli
Klebsiella pneumoniae
Klebsiella spp., not K. pneumoniae
Citrobacter spp.
Enterobacter spp.
Other Enterobacteriaceae
Number of patients with first positive carbapenem-resistant Enterobacteriaceae isolation, by week of specimen collection
0
5
10
blaKPC-positive species isolated from environmental samples
Dec 2013
Jan 2014
Feb 2014
Mar 2014
Apr 2014
May 2014
Jun 2014
Jul 2014
Aug 2014
Sep 2014
Oct 2014
Nov 2014
Dec 2014
Jan 2015
Feb 2015
Mar 2015
Apr 2015
May 2015
Jun 2015
Jul 2015
Aug 2015
Sep 2015
Oct 2015
Nov 2015
Dec 2015
Jan 2016
Feb 2016
Mar 2016
Apr 2016
May 2016
Jun 2016
Jul 2016
Aug 2016
Sep 2016
Oct 2016
Nov 2016
Dec 2016
Jan 2017
B.
Event/Intervention
Environmental screening
Weekly patient screening
Twice weekly patient screening
Plumbing replacement
Staff cohorting
Patient cohorting
Ward 4 closures
Ward 3 closures
Outbreak alert by IPC team
Dec 2013
Jan 2014
Feb 2014
Mar 2014
Apr 2014
May 2014
Jun 2014
Jul 2014
Aug 2014
Sep 2014
Oct 2014
Nov 2014
Dec 2014
Jan 2015
Feb 2015
Mar 2015
Apr 2015
May 2015
Jun 2015
Jul 2015
Aug 2015
Sep 2015
Oct 2015
Nov 2015
Dec 2015
Jan 2016
Feb 2016
Mar 2016
Apr 2016
May 2016
Jun 2016
Jul 2016
Aug 2016
Sep 2016
Oct 2016
Nov 2016
Dec 2016
Jan 2017


A.
Patient on MHC wards
P1
n=4
P2
n=90
P3
n=0
P4
n=6
Number of patients with first isolation of CR-E. coli
0
2
4
6
8
10
12
2010
2011
2012
2013
2014
2015
2016
2017

B.
Patient on wards 45/46
Patients not on MHC wards or wards 45/46
P1
n=49
n=150
P2
n=10
n=108
P3
n=4
n=28
P4
n=9
n=55
Number of patients with first isolation of CR-E. coli
0
2
4
6
8
10
12
2010
2011
2012
2013
2014
2015
2016
2017


C.
Incidence rate ratio for isolation of CR-E. coli relative to P2
Incidence rate ratio for isolation of CR-K. pneumoniae relative to P2
Incidence rate ratio for isolation of any CRE relative to P2
3.00
2.00
1.00
0.70
0.50
0.30
0.20
0.10
0.03
0.01
0.003
0.001
P1
P3
P4
Time period examined
Location
MHC
Other wards


Tree scale: 0.001
ST131
ST405
ST69
ST401
ST410
ST607
ST216
Strain-A1
Strain-A2
OUTER RING legend
Environmental isolate
Environmental isolate - post drain replacement
Patient isolate - post drain replacement
pKPC-CAD2
pKPC-CAD1 (or pCAD3)
Tn4401a-AGTTG
Tn4401a-ATTGA
Tn4401a-TAATA
Other Tn4401a
MHC
CMFT ward 45/46
CMFT other wards
Other regional hospitals
INNER RING legend
First carbapenem-resistant E. coli from patient
Regional study isolate
Unlinked epidemiological information
Sequential carbapenem-resistant E. coli from patient
Environmental isolate
Phenotypically carbapenem-susceptible
No blaKPC identified on sequencing


A.
pCAD3
pKPC_CAD1
pKPC_CAD2
pKPC-272
Maryland, USA
sink drain
Jan 2012
Tn4401
bla_{KPC}
B.
CMFT other wards
CMFT wards
45 and 46
(geratology)
Wards 3 and 4
(MHC)
Other regional
hospitals
2011
2012
2013
2014
2015
2016
E. coli ST
Plasmid type
Other ST
Other
ST216
pKPC_CAD2/pCAD3
(geratology outbreak 2012)
ST216
Other
ST216
pKPC_CAD1
(MHC outbreak 2015)
Other ST
pKPC_CAD2
ST607
pKPC_CAD2
ST401
pKPC_CAD2
ST409
pKPC_CAD2/pCAD3
ST607
Other
ST401
Other