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1 **A large, refractory nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase**
2 **(KPC)-producing *Escherichia coli* demonstrates carbapenemase gene outbreaks**
3 **involving sink sites require novel approaches to infection control**

4
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37

38 **Running title:** *bla*_{KPC}-*E. coli* outbreak in Manchester, UK

39

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48 **ABSTRACT**

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

55

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

62

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

70

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

- 73 recurred rapidly following this intervention. The large numbers of cases and persistence of
- 74 *bla_{KPC}* in *E. coli*, including pathogenic lineages, is a concern.

75 INTRODUCTION

76 Carbapenem-resistant *Enterobacteriaceae* (CRE) are a global public health threat(1). Major
77 carbapenemases include the metallo-beta-lactamases, some oxacillinases and the *Klebsiella*
78 *pneumoniae* carbapenemase (KPC, encoded by *bla*_{KPC}), one of the commonest
79 carbapenemases globally(2). Transfer of carbapenemase genes on mobile genetic elements
80 has resulted in the rapid, inter-species dissemination of carbapenem resistance(3, 4). Since
81 few therapeutic options remain for CRE infections(5, 6), effective control is critical.

82

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

88

89 CRE detections in England have increased since 2008(9), and are approximately ten times the
90 national average in Greater Manchester(10). Central Manchester University Hospitals NHS
91 Foundation Trust (CMFT) has experienced an on-going multi-species *bla*_{KPC}-associated CRE
92 outbreak since 2009. Intensive Infection Prevention and Control (IPC) measures, in line with
93 national and international recommendations(11-13), have been implemented in response.

94

95 In 2015, a sudden increase in cases of faecal colonisation with KPC-producing *E. coli* (KPC-
96 EC) was detected in the Manchester Heart Centre (MHC) at the Manchester Royal Infirmary
97 (MRI; part of CMFT). We retrospectively investigated the genomic epidemiology and
98 evidence for nosocomial transmission of KPC-EC and KPC plasmids isolated from patients

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

101

102 **RESULTS**

103 **High prevalence of CRE colonisation in the MHC**

104 Between 01/Apr-30/Dec/2014, 23 new CRE-colonised individuals were detected on the
105 MHC, including two with *E. coli* (Fig.1A). A CRE outbreak was declared on 02/Jan/2015
106 when six new CRE-colonised individuals were identified (four with *bla*_{KPC}, two with *bla*_{NDM};
107 no *E. coli*). Consequently, intensified IPC measures were implemented (Table S1; Fig.1B),
108 and W3/W4 were closed (06/Jan/2015), terminally cleaned (hypochlorite), and
109 decontaminated (hydrogen peroxide vapour). W3 was re-opened on 11/Jan/2015 and W4 on
110 23/Jan/2015; high-risk patients (CRE previously detected/history of hospitalisation abroad or
111 in UK hospital with known CRE transmission in past 12 months) were screened; CRE-
112 positive patients were transferred to a cohort ward or, if they required cardiac monitoring, to
113 side-rooms.

114

115 By January 2015, CMFT was operating a Trust-wide CRE screening program (>110
116 screens/day; Table S2). Between 01/Sep/2014-30/Dec/2014, screening transitioned from
117 culture- to PCR-based methods: during this period 16,612 samples from 7,239 inpatients
118 were screened using either culture (n=9,808), or PCR+culture (n=6,804), with an overall
119 CRE prevalence of 3.8% (438 positive samples, 272 patients). Molecular mechanism data for
120 135/163 (83%) PCR-positives indicated *bla*_{KPC} accounted for most carbapenem resistance
121 (97%).

122

123 **KPC-*E. coli* outbreak despite IPC interventions**

124 Following the implementation of enhanced IPC activity, there was a further sharp increase in
125 the number of CRE-colonised patients detected from 09/Mar/2015 (Fig.1A; CR-*E. coli* and
126 other species, mostly *bla*_{KPC}, a few *bla*_{NDM}). W3 was again closed to admissions
127 (11/Mar/2015-28/Mar/2015) and environmental decontamination repeated; the following
128 week W4 was closed after detection of additional CRE-colonised patients (Figs.1A, 1B).
129 From 01/April/2015 KPC-EC predominated in the outbreak (Fig. 1A).

130

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

139

140 **Carbapenem-resistant *E. coli* cases in CMFT**

141 CR-*E. coli* had been isolated in CMFT prior to the 2015 MHC outbreak, with 514 CR-*E. coli*
142 cases (considering first positives by patient from clinical/screening isolates, 2010-2016
143 inclusive), and including a separate outbreak on the geratology wards (W45/46) in late 2012
144 (Figs.2A,2B). Of these, 434 cases were detected on \geq day 2 of admission, and a further 80 on
145 day 0-1 of admission. Case peaks were not related to screening policy changes/rates (Fig.S6).
146 CR-*E. coli* were almost invariably detected from rectal screening (420/434 cases, 97%).

147

148 **Environmental sampling yielded CRE from sinks/drains**

149 Intermittent environmental sampling was undertaken to identify potential reservoirs. Overall,
150 927 samples from 833 sites were taken 09/Apr-17/Nov/2015; 355 (38%) samples from 333
151 (40%) sites were from W3/W4, and the remainder from eleven other wards. 850 samples
152 were from sink/drain/shower/bath sites, 18 from toilets/hoppers/sluices, and 33 from high-
153 touch sites (including keyboards, door handles, sponges etc.; labelling unclear for 26
154 samples). Eighty-five samples (9%) and 72 sites (9%) were CRE-positive (26/355 samples
155 [7%], 21/333 sites [6%] on W3/4). CRE-positive sites included: shower drains (n=19), sink
156 taps (n=7); sink drain tailpieces (n=10); sink drain strainers (n=8); sink trap water (n=1);
157 toilet bowls (n=1); other (n=26). Common isolates cultured included: *Klebsiella* spp. (n=34),
158 *Enterobacter* spp. (n=25), and *E. coli* (n=11) (Fig.1A). All CRE-positive cultures were from
159 wastewater/plumbing-associated sites; no other sites tested were CRE-positive.

160

161 Of ten sites yielding 11 KPC-EC isolates, five were in the W3/W4 kitchen (14-18/May/2015
162 [n=4], 10/Sep/2015 [n=1]), one a W4 staff sink (14/May/2015), and four from kitchen
163 sinks/drains on wards 31/32 (sampling in response to a separate ward 31/32 outbreak, 12-
164 17/Nov/2015). W3/W4 sink-specific interventions included sink trap replacement for CRE-
165 colonised sinks (16/Apr/2015, 31/Jul/2015, 11/Aug/2015) and horizontal pipework cleaning
166 with a brush to try and remove biofilm (11/Aug/2015).

167

168 **Cardiac service relocation and decline in CRE colonisation incidence**

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

174 <https://www.aqua-free.com/en/gb/medical-water-hygiene/products/medical->
175 [application/produkt/Ressort/product/hygienesiphon/](https://www.aqua-free.com/en/gb/medical-water-hygiene/products/medical-application/produkt/Ressort/product/hygienesiphon/)).

176

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

192

193 **Genomic epidemiology of KPC-EC**

194 268 clinical and environmental CR-*E. coli* isolates were sequenced. These included 82
195 isolates from the MHC (2015-2016 [16 environmental]), 36 from W45/W46 (2010-2016),
196 109 from other CMFT wards/units, and 41 from other regional hospitals (Table S3). Nine
197 isolates were *bla*_{KPC}-negative on sequencing; five of these contained *bla*_{OXA-48}, one *bla*_{OXA-181},
198 and one *bla*_{NDM-5}, with no known carbapenem resistance mechanisms identified in the

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

203

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

208

209 *ST216 KPC-EC*

210 The ST216 KPC-EC group (n=126; 9,118 variable sites; one *bla*_{KPC}-negative isolate
211 [H134880341]) was represented by two main genetic sub-groups consisting of 112 isolates
212 (main outbreak strain, denoted strain-A1 in Fig.3; ≤65 SNVs between isolates in this cluster,
213 2012-2016), and 12 isolates respectively (secondary outbreak strain, strain-A2 in Fig.3, ≤25
214 SNVs between isolates in this cluster; >7,800 SNVs divergent from strain-A1 isolates, 2012-
215 2015). Although the SNV-based distances between strains-A1 and -A2 were large, review of
216 the ClonalFrameML output suggested these differences represented a single “mega”-
217 recombination event affecting ~1Mb of the genome (Fig.S7).

218

219 All but three ST216 isolates carried *bla*_{KPC-2} in a *Tn4401a* transposon(14), typically
220 associated with high-level *bla*_{KPC} expression(15), and flanked by a 5-bp target site
221 duplication, AGTTG, previously only observed with the *Tn4401b* isoform in an isolate from
222 Colombia (Fig.3, Table S3). This relatively unique transposon-flanking sequence unit was
223 also observed in other lineages within CMFT (e.g. ST401, Fig.3). However, plasmid and

224 resistance gene profiles varied considerably, even to some extent within the ST216 KPC-EC
225 outbreak strains (Figs.3, S8). Overall, these results demonstrated clonal expansion of specific
226 KPC-EC strains, with significant accessory genome mobility. Most notable was the
227 emergence and persistence of ST216 KPC-EC strain-A1, isolated from patients and the
228 environment over four years, and causing outbreaks on W45/W46 (2012) and the MHC
229 (2015).

230

231 Long-read sequencing demonstrated that the ST216 KPC-EC strain-A1 isolate H124200646
232 (W46, 2012) contained two plasmids, pKPC-CAD2 (307kb; IncHI2/HI2A; *bla*_{KPC} present)
233 and pCAD3 (152kb; IncFIB/FII; *bla*_{KPC} absent). 83% of pKPC-CAD2 was highly similar
234 (99% sequence identity) to pKPC-272 (282kb, *E. cloacae*, GenBank accession CP008825.1),
235 identified in a sink drain at the National Institutes of Health Clinical Centre, Maryland, USA,
236 2012(16). In contrast, the other long-read sequence, H151860951 (W4, April 2015), also an
237 ST216 KPC-EC strain-A1 isolate, contained a *bla*_{KPC}-plasmid pKPC-CAD1 (200kb;
238 IncFIB/FII), which had 99% sequence identity over 76% of its length to pCAD3, together
239 with a 48kb contiguous region including *bla*_{KPC} that was 99% identical to part of pKPC-
240 CAD2 (Fig.4A). These results suggest the evolution of a *bla*_{KPC} plasmid similar to pKPC-272
241 in CMFT within an ST216 KPC-EC strain-A from 2012-2015, including recombination
242 between pKPC-CAD2 and pCAD3 giving rise to pKPC-CAD1.

243

244 Although plasmid typing based on mapping short-read data to plasmid references should be
245 interpreted cautiously, sequence comparisons with the outbreak plasmids pKPC-CAD1 and
246 pKPC-CAD2 were consistent with the emergence of pKPC-CAD1 and its domination within
247 ST216 KPC-EC strain-A post-2014; and exchange of pKPC-CAD1/pKPC-CAD2/pCAD3
248 with other *E. coli* STs (Fig.3; Fig.4B).

249

250 *Environmental CRE isolates*

251 Thirty environmental carbapenem-resistant *Enterobacteriaceae* isolates from W3/W4 were
252 sequenced, 27 isolated prior to the plumbing replacement, and 16 of which were CR-*E. coli* ,
253 as described above (13 prior to plumbing replacement). 11/16 *E. coli* were ST216 KPC-EC
254 (ten strain-A1, one strain-A2), isolated on eight separate days (in March, May, September
255 2015, February 2016), and consistent with transmission between patients and the
256 environment (Fig.3), and persistence/reintroduction following plumbing replacement. The
257 other 14 isolates represented diverse KPC-CRE, including: *K. pneumoniae* (n=7), *Citrobacter*
258 *freundii* (n=4), *Klebsiella oxytoca* (n=1), *Enterobacter cloacae* (n=1) and *Kluyvera*
259 *intermedia* (n=1). The KPC plasmids in these KPC-CRE likely included the outbreak
260 plasmids pKPC-CAD1 and pKPC-CAD2, pKpQIL, and others, consistent with the inter-
261 species transfer of a diverse set of *bla*_{KPC} plasmids.

262

263 **DISCUSSION**

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

272

273 Environmental sampling on W3/W4 confirmed that sinks/drains were colonised by multiple
274 CRE, including the ST216 KPC-EC strains-A1/A2 and other CRE containing the outbreak
275 KPC plasmids (pKPC-CAD1, pKPC-CAD2), potentially representing a persistent reservoir
276 between patient-associated outbreaks, and plausibly explaining why this large outbreak was
277 refractory to standard IPC bundles. Supporting this, the incidence of new CR-*E. coli*
278 detections declined substantially after ward plumbing replacement and temporary relocation
279 of patients (Figs.1A, 2A, 2C), consistent with a major contribution from the ward
280 environment. However, after W3/W4 reopened the environment was rapidly re-contaminated,
281 including with ST216 KPC-EC strain-A1, and CRE were again detected in patients,
282 suggesting that this type of intervention has limited durability. National and international
283 guidelines on CRE management recommend rectal screening, strict contact precautions,
284 isolation/cohorting of cases, and antimicrobial stewardship to limit transmission(12, 13, 17),
285 all measures already implemented in CMFT. Current guidelines do not address the control of
286 large, persistent outbreaks, or advise on the sampling and management of environmental
287 reservoirs, and there is limited evidence in support of any given measure(18). It is unclear
288 why a particular strain of KPC-*E. coli* predominated in the outbreak described, as opposed to
289 other CRE contemporaneously found in the environment - differences in gastrointestinal
290 colonisation ability of species, or an unidentified point source could be potential hypotheses.
291
292 The response to this outbreak caused major disruption to the hospital and regional cardiac
293 services. Given that almost all cases represented colonisations and not infections, the risks of
294 associated delays in cardiac interventions were debated, although the impact of these were
295 not formally quantified. The estimated cost of CRE to CMFT in the first 8 months of 2015
296 was £5.2m(19), and the MHC outbreak contributed significantly to this, with ~£240,000
297 spent on the W3/W4 plumbing replacement.

298

299 The study has several limitations, including its observational nature, with only a year of
300 follow-up after the W3/W4 plumbing replacement. Limited environmental sampling may
301 have meant that the extent of contamination and diversity of CRE in environmental niches
302 was underestimated. Environmental sampling was restricted to wards on which CRE
303 outbreaks were detected and focused predominantly on sink/drain sites (as initial sampling
304 suggested these were most heavily contaminated); however, component parts of each sink
305 drainage system were not sampled consistently due to resource issues and so relative CRE
306 isolation prevalence from any given site type needs to be interpreted with caution. We only
307 sequenced single isolates cultured from individuals at any given time-point due to resource
308 limitations, and may therefore have underestimated the CRE strain diversity within patients.
309 Other non-*E. coli* *Enterobacteriaceae* were not comprehensively sequenced, possibly
310 underestimating dissemination of pKPC-CAD1 and pKPC-CAD2; however, even our limited
311 sequencing of CREs from the environment in 2015 identified these plasmids (and other KPC
312 plasmids) in multiple species. Although genetic overlap between environmental and patient
313 isolates was consistent with transmission between these compartments (Fig.3), the numbers
314 were too small to infer directionality. Of the two predominant KPC plasmid types present
315 within the ST216 KPC-EC strain-A1 outbreak clone, one (pKPC_CAD2) was transferred to
316 multiple *E. coli* STs (Figs.3, 4B), and another (pKPC_CAD1) may have contributed to the
317 clone's success from 2014 (Fig.4B), although the genetic/biological mechanisms
318 underpinning this have not been explored.

319

320 Our experience highlights the limited evidence for managing large CRE outbreaks including
321 environmental sampling protocols and interventions, despite numerous centres reporting
322 similar experiences with wastewater sites acting as CRE reservoirs(18, 20-23). Widespread

323 colonisation with KPC-EC is a concern, as *E. coli* is a common gastrointestinal colonizer and
324 cause of infection, and any stable association between *bla*_{KPC} and *E. coli*, particularly in
325 pathogenic lineages such as ST131 (Fig.3), represents a significant clinical and transmission
326 threat. Although our analyses focused on CRE, similar wider environmental contamination
327 and dissemination of carbapenem-susceptible *Enterobacteriaceae* seem plausible. A more
328 robust evidence base delineating transmission networks (including initial contamination of
329 sink sites), drivers and effective control measures (including differential impacts of
330 decontamination methods on particular species/strains), is needed to minimize the financial,
331 clinical and social impacts of CRE outbreaks.

332

333 **MATERIALS and METHODS**

334 **Setting**

335 CMFT is one of the largest hospital trusts in northwest England. The MHC manages >10,000
336 patients/year, and in 2015 included two 28-bedded inpatient wards (Wards 3 [W3] and 4
337 [W4]), an acute facility (Ward 35), intensive care unit, and cardiac catheter laboratory. Both
338 W3 and W4 comprised three bays, four single-patient side-rooms, and a shared kitchen (Figs.
339 S1A, S1B).

340

341 **IPC measures**

342 CRE screening/IPC measures, based on UK guidelines(11), were implemented Trust-wide
343 from mid-2014. Enhanced measures were introduced in April 2015 in response to the MHC
344 KPC-EC outbreak (Table S1). In addition, W3/W4 (where most KPC-EC cases were
345 observed) were closed to replace plumbing infrastructure back to the drainage stacks (Fig.
346 S2) from September 2015. Staff screening was not undertaken, consistent with national
347 guidelines(11).

348

349 **Patient CRE screening**

350 Rectal swabs were screened for CRE using selective chromogenic agar (ChromID CARBA,
351 Biomerieux; published sensitivity: 89-100%, specificity: 95%(24-26)) to August 2014, and
352 the Cepheid Xpert Carba-R assay (published sensitivity: 97-100%, specificity: 99%(27, 28))
353 from August 2014, alongside an in-house multiplex PCR (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}) from
354 November 2014. The Cepheid assay was used on specimens from patients with admissions to
355 the Trust in the last 12 months, those admitted from overseas, or those due to be transferred
356 to a district general hospital (to facilitate transfer planning). All other samples were tested
357 using the multiplex PCR. Species identification of isolates was performed using MALDI-
358 TOF mass spectrometry (Bruker).

359

360 **Epidemiological analyses**

361 CMFT electronic bacteriology records were linked on NHS number to patient administration
362 data (01/Jan/2010-01/Jan/2017) and anonymised, and the first-CRE-positive test result per
363 patient (rectal screening or clinical specimen) was considered in the evaluation of CRE
364 incidence trends. Trends and the impact of IPC interventions were analysed retrospectively.

365

366 As CMFT CRE screening rates changed over time in response to national guidance and local
367 IPC interventions, and a key aim was to specifically evaluate the impact of ward closure and
368 a radical plumbing intervention in the MHC on CRE acquisition rates, we considered CRE
369 detection rates in four periods delineated by three time points: the implementation of national
370 CPE IPC policy in mid-2014 (which substantially increased the number of screens
371 performed), the beginning of the MHC-specific intervention (patient relocation and plumbing
372 infrastructure replacement on W3/W4), and the end of the MHC intervention.

373

374 First-CRE positive screens were used as a pragmatic proxy for CRE acquisition (i.e. a
375 “case”), given that 89% of patients first-CRE positive on the MHC had a negative rectal
376 screen within the preceding 14 days (79% within 7 days; Figs S3-5). Information on specific
377 carbapenemase mechanism was not consistently available for all isolates, precluding our
378 ability to perform these analyses specifically by carbapenemase gene family (Table S2).

379

380 We tested the hypothesis that CRE acquisitions (reflected by first CRE-positive screens)
381 changed on the MHC more than other hospital wards following the W3/W4 closure/plumbing
382 intervention using negative binomial regression models for the weekly counts of first (per
383 person) CRE detection ≥ 2 days post-admission (i.e. cases), using weekly numbers of persons
384 screened ≥ 2 days post-admission as an offset (i.e. adjusting for screening rates, and counting
385 each patient as screened as long as they had one or more screens per week). Models were
386 fitted (R v3.4.1) for CRE, carbapenem-resistant *E. coli* (CR-*E. coli*), and carbapenem-
387 resistant *K. pneumoniae* (CR-*K. pneumoniae*). We included period and ward location (MHC
388 versus other wards) as independent variables, plus interaction terms between period and
389 location (details in Supplementary Methods).

390

391 **Environmental sampling and sample processing**

392 In 2015, environmental samples were taken from ward sites using charcoal swabs, and
393 cultured on ChromID CARBA (18 hours, 37°C). After January 2016, ~20mls of wastewater
394 was aspirated from sink P-traps, shower drains or toilets. Aspirates were centrifuged at
395 4000rpm for 10mins, 15mls of supernatant were discarded, and the pellet was re-suspended
396 in the remaining 5mls. One ml of sample was then incubated aerobically overnight (~37°C)
397 in 5mls trypticase soy broth with an ertapenem disc; the multiplex PCR (as above) was

398 performed on broths to identify *bla*_{KPC}-positive samples for subsequent culture on ChromID
399 CARBA. Environmental sampling prior to January 2016 was not systematic; after January
400 2016, 75 wastewater sites on W3/W4 were sampled fortnightly on rotation (half of the sites
401 one week and half the next). These sites included toilets, sink basins and sink drains.

402

403 **Genome sequencing and sequence data analysis**

404 To provide genetic context for the outbreak, we sequenced retrievable, archived KPC-EC
405 patient and environmental isolates from CMFT, and patient isolates collected for regional
406 public health surveillance (Supplementary Methods; Table S3). We also sequenced a small
407 subset of non-*E. coli* environmental CRE that had been stored (n=14) ad hoc as part of
408 outbreak sampling prior to the plumbing replacement.

409

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

417

418 *In silico* species identification was performed using Kraken(29). Illumina reads were then
419 mapped to species-specific references (*E. coli* CFT073 [AE014075.1], and the ST216
420 reference H151860951) and base-calling performed as previously(30). *De novo* assembly was
421 performed using SPAdes (v3.6)(31) and resistance gene, *bla*_{KPC} plasmid and Tn4401 typing
422 using BLASTn and mapping-based approaches (Supplementary Methods; Table S3).

423

424 2D-reads were extracted from MinION sequence data using poretools(32); hybridSPAdes(31)
425 and Canu(33) were used to generate *de novo* hybrid assemblies from MinION+Illumina data
426 (Supplementary Methods). PacBio sequence data were *de novo* assembled using HGAP3(34).
427 *E. coli* phylogenies were reconstructed using IQTree(35) and ClonalFrameML(36), and
428 visualised in iTOL(37) (Supplementary Methods).

429

430 **Ethical approval**

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

433

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444

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464

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466

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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, Tn4401

631 type and outbreak plasmid types. Earliest available sequences per patient are denoted “first
632 carbapenem-resistant *E. coli* from patient” if the stored isolate collection date was ≤ 7 days
633 from the first isolation date in the TRACE database, or “sequential carbapenem-resistant *E.*
634 *coli* from patient” if the stored isolate date was after this. KPC-EC isolates from a Public
635 Health England (PHE) project sequencing the first ten KPC-*Enterobacteriaceae* from
636 hospitals in northwest England (2009-2014) are denoted “regional study isolates”.
637 “Environmental isolates” denote KPC-EC cultured during an initial environmental prevalence
638 survey on W3/W4 (10/Mar/2015); any KPC-EC isolated as part of subsequent, intermittent
639 IPC-associated environmental sampling (09/Apr/2015-17/Nov/15); and isolates available at
640 the time of analysis from environmental and patient samples from a separate, on-going study
641 (commenced January 2016).

642

643 **Figure 4.A.** Alignments of Manchester Heart Centre (MHC) outbreak 2012 KPC plasmid
644 pKPC-CAD2 (W45/46; Tn4401a+*bla*_{KPC}) and the 2015 MHC KPC plasmid pKPC-CAD1
645 (Tn4401a+*bla*_{KPC}), highlighting the recombination of the Tn4401a+*bla*_{KPC}-harbouring 48kb
646 segment from pKPC-CAD2 with pCAD3 to generate pKPC-CAD1. Regions of sequence
647 homology are represented by salmon-pink links drawn between alignments. pKPC-272
648 (GenBank accession CP008825.1), a plasmid identified in an isolate in a sink drain at the
649 National Institutes of Health Clinical Centre, Maryland, USA, 2012, demonstrates significant
650 sequence homology with pKPC-CAD2. **B.** Incidence plot of different *E. coli* STs and likely
651 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 <i>Enterobacteriaceae</i> (number of cases=3,086)			Carbapenem-resistant <i>E. coli</i> (number of cases=502)			Carbapenem-resistant <i>K. pneumoniae</i> (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
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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
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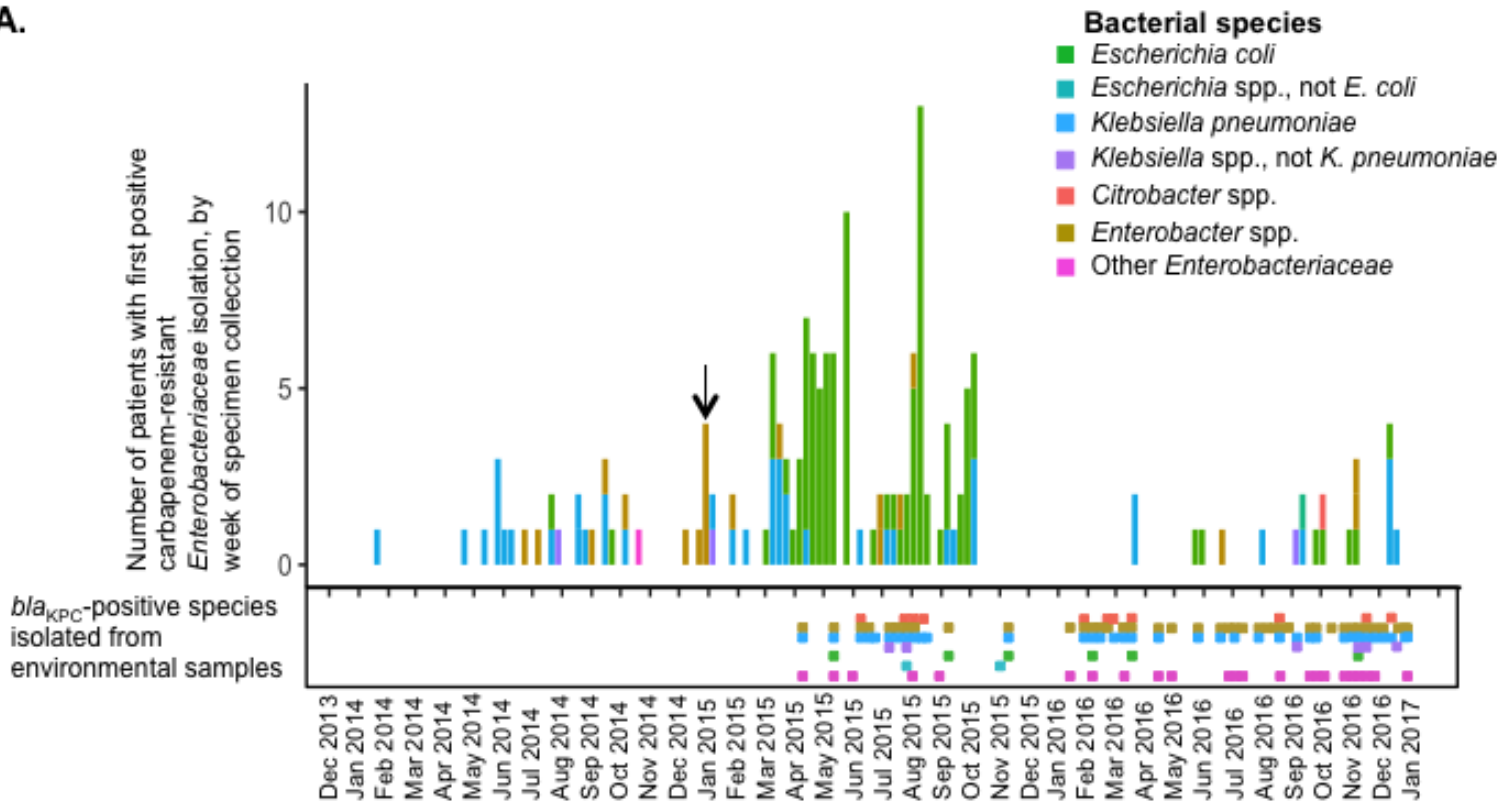
Heterogeneity between reduction in

MHC vs other location

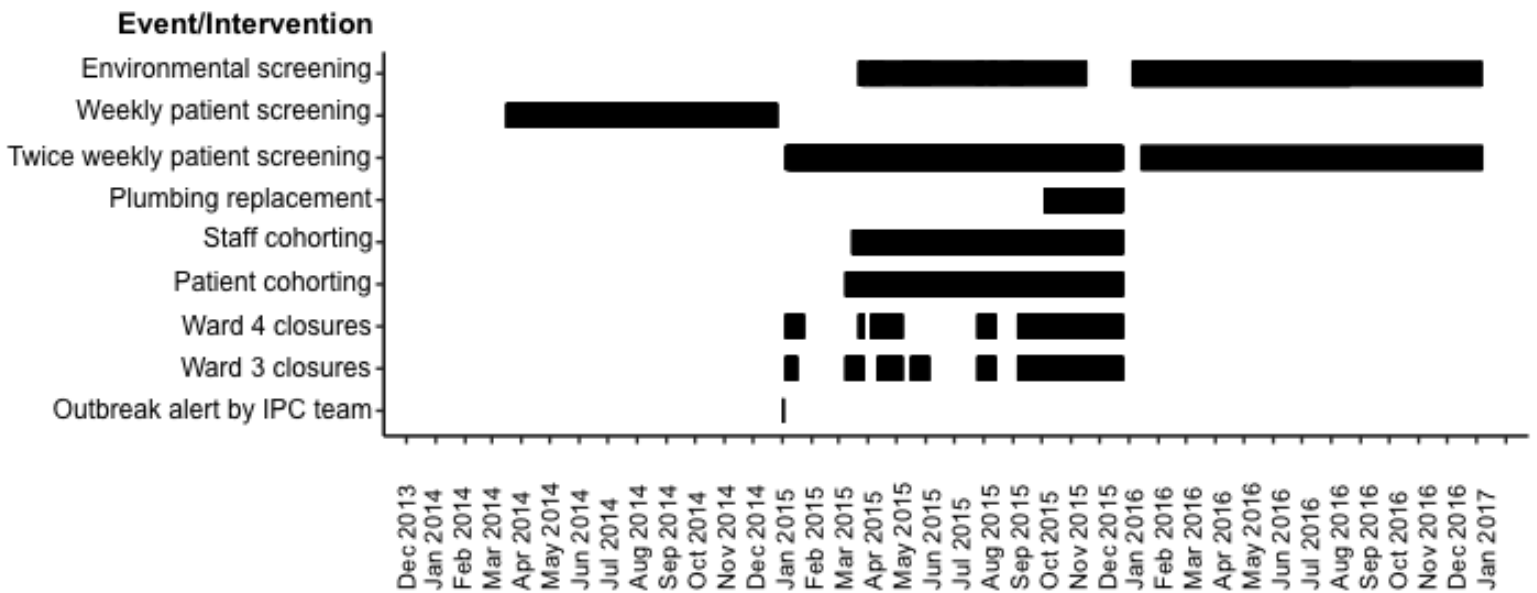
Week 03 2010 to week 26 2014 (P1)			<0.001			0.001			0.098
Week 40 2015 to week 02 2016 (P3)			-			-			-
Week 03 2016 to week 52 2016 (P4)			<0.001			0.003			0.31

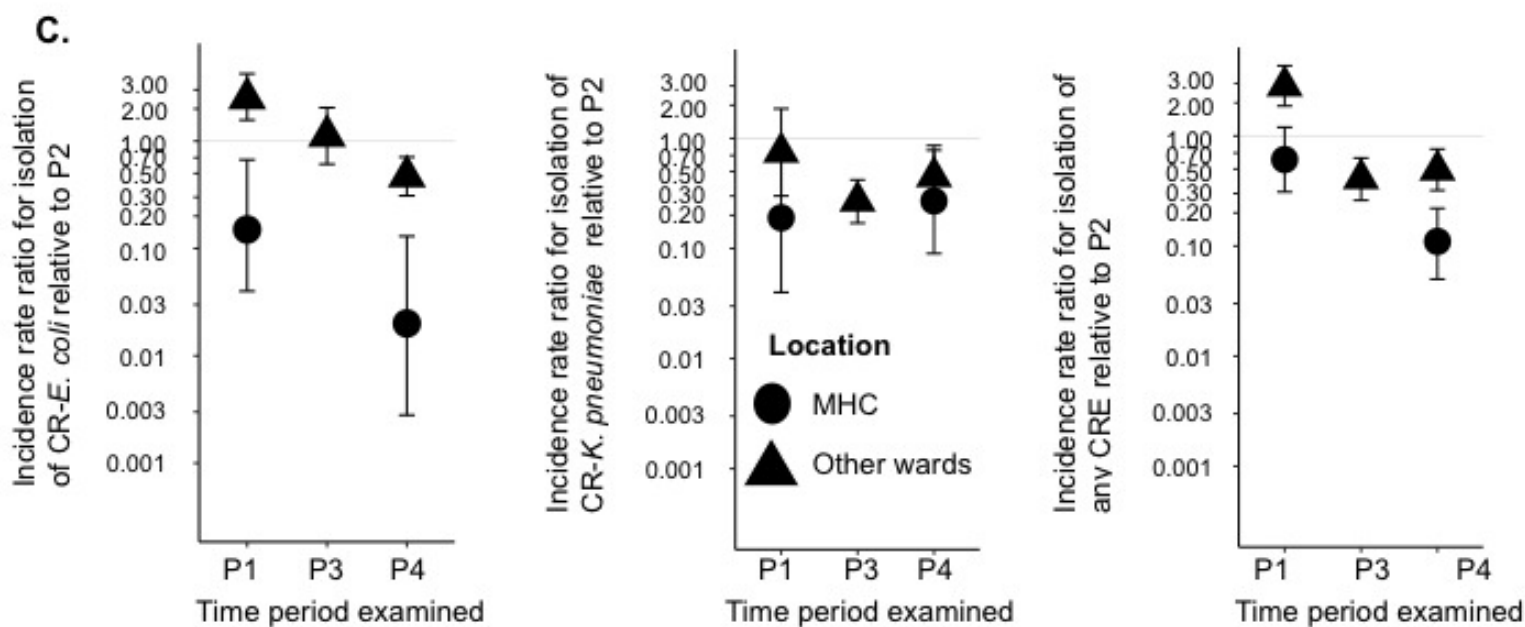
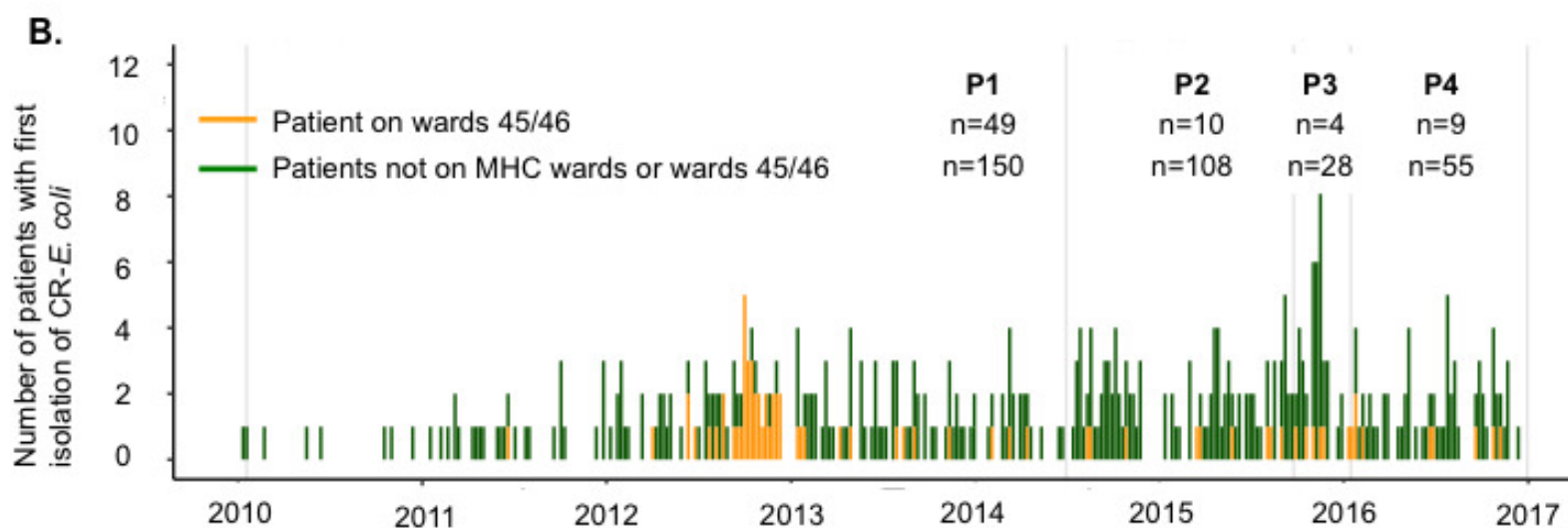
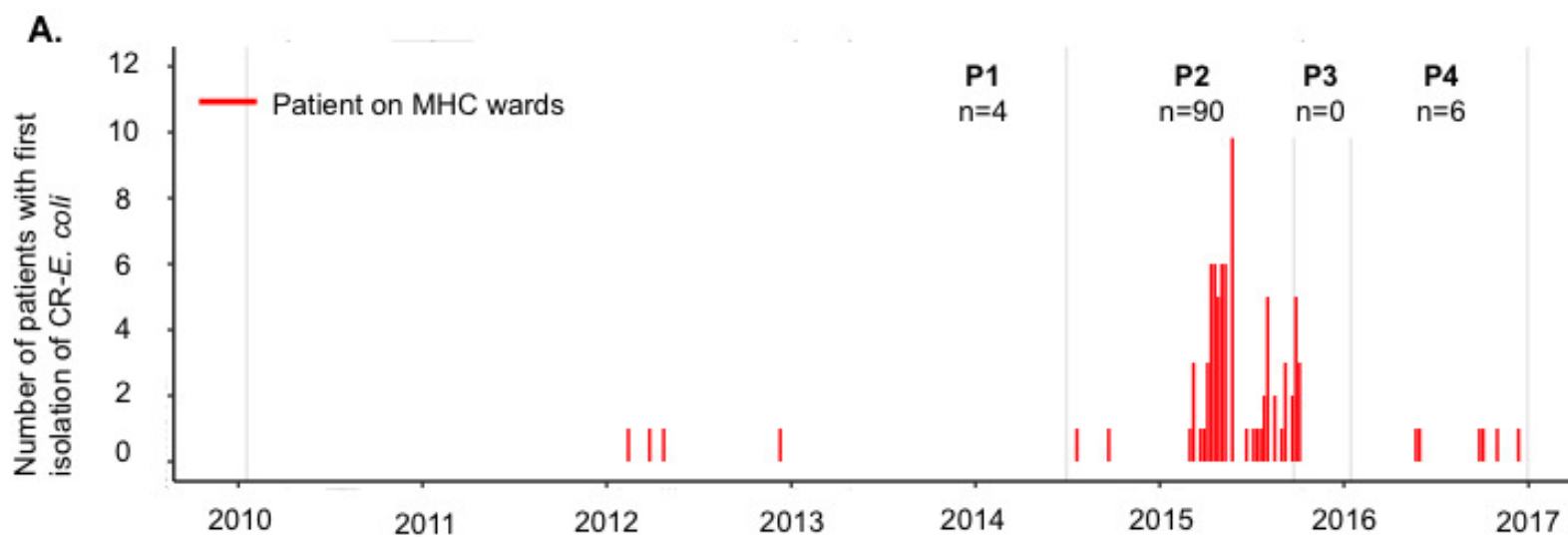
658 * P2 chosen as reference period because of change in screening policy between P1 and P2 (Table S2, Fig.S6), meaning that a greater incidence
659 would be expected in P2 due to more patients being screened every week.

A.



B.





Tree scale: 0.001

