1    **Extended-spectrum β-lactamase genes traverse the *Escherichia coli***

2    **populations of ICU patients, staff and environment.**

3

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19   **Keywords:** *E. coli*, ICU, ESBL, plasmid, transposition

20    **Abstract**

21    Over a three-month period, we monitored the population of extended-spectrum β-lactam-

22    resistant *Escherichia coli* (ESBL-EC) associated with the patients, staff and environment of an

23    intensive care unit (ICU) in Guangzhou, China. Thirty-four clinical isolates were obtained

24    from the same hospital 12 months later. A total of 165 isolates were characterised and

25    whole-genome sequenced, with 24 isolates subjected to long-read sequencing. The diverse

26    population included representatives of 59 different sequence types (STs). ICU patient and

27    environmental isolates were largely distinct from staff isolates and clinical isolates. We

28    observed five instances of highly similar isolates (0-13 core-gene SNPs) being obtained from

29    different patients or bed unit environments. ESBL resistance in this collection was largely

30    conferred by $bla_{CTX-M}$ genes, which were found in 96.4% of all isolates.  The contexts of

31    $bla_{CTX-M}$ genes were diverse, situated in multiple chromosomal positions and in various

32    plasmids. We identified $bla_{CTX-M}$-bearing plasmid lineages that were present in multiple STs

33    across the surveillance, staff and clinical collections. Closer examination of IS$Ecp1$-$bla_{CTX-M}$

34    transposition units shed light on the dynamics of their transmission, with evidence for the

35    acquisition of chromosomal copies of $bla_{CTX-M}$ genes from specific plasmid lineages, and for

36    the movement of $bla_{CTX-M-55}$ from a ST1193 chromosome to a small mobilisable plasmid. A

37    carbapenem-resistant ST167 strain isolated from a patient that had been treated with

38    meropenem and piperacillin-tazobactam contained seven copies of $bla_{CMY-146}$, which

39    appears to have been amplified by IS*1*. Our data revealed limited persistence and

40    movement of ESBL-EC strains in the ICU environment, but we observed circulating plasmid

41    lineages playing an essential and ongoing role in shaping the cephalosporin-resistance

42    landscape in the population examined.

43    **Impact statement**

44    ESBL resistance significantly impacts clinical management of *E. coli* infections in hospitals

45    globally. It is important to understand the structures of ESBL-EC populations carried by

46    hospital patients and staff, their capacity to persist in hospital environments, and the

47    dynamics of mobile genes that drive the spread of ESBL resistance. In our three-month

48    study, ESBL-EC strains found in the ICU environment were strongly associated with patient

49    carriage, but distinct from strains found in staff. However, plasmid lineages carrying $bla_{CTX-M}$

50    genes were found across the ICU populations and in a collection of clinical isolates obtained

51    one year later. By examining their content and contexts, we have traced the recent histories

52    of chromosomal and plasmid-borne IS*Ecp1*-$bla_{CTX-M}$ transposition units in the ICU

53    population. This allowed us to implicate specific plasmid lineages in the acquisition of

54    chromosomal $bla_{CTX-M}$ genes, even when the plasmids were no longer present, and to detect

55    recent transposition of $bla_{CTX-M-55}$ from a chromosome to a mobilisable plasmid. Similar high-

56    resolution approaches to the study of mobile genetic elements will be essential if the

57    transmission routes associated with the spread of ESBL resistance are to be understood and

58    subjected to interventions.

59

60    **Data summary**

61    Sequencing reads are available under NCBI BioProject accession PRJNA907549. The 91

62    complete plasmid sequences generated in this study are in a supplementary file called

63    pDETEC_collection.fa.

64 **Introduction**

65 *Escherichia coli* occupies a niche in the human gastrointestinal tract that makes it an

66 important vehicle for mobile genes that confer resistance to clinically-relevant antibiotics.

67 Some clones from the vastly diverse *E. coli* population can cause human infections,[1] so the

68 importance of antibiotic resistance gene carriage by the species is twofold: infections

69 caused by antibiotic-resistant *E. coli* are more difficult to treat, and antibiotic resistance

70 genes carried by human-associated *E. coli* can be transferred to other Gram-negative

71 pathogens. Extended-spectrum β-lactam (ESBL)-resistant *E. coli* (ESBL-EC) usually carry one

72 or more of the various horizontally-acquired β-lactamase (*bla*) genes that can be located in

73 various chromosomal positions or in plasmids. The $bla_{CTX-M}$ genes are some of the most

74 clinically important, and have been detected globally in *E. coli* and other members of the

75 Enterobacterales.[2] In China, $bla_{CTX-M-55}$ has been increasing in prevalence, and in recent years

76 has overtaken $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$ as the most common ESBL resistance gene seen in

77 ESBL-EC associated with human infections.[3,4]

78

79 Dissemination of ESBL resistance genes through global bacterial populations has been

80 facilitated by mobile genetic elements (MGEs).[5] Plasmid-mediated intercellular transfer

81 plays an obvious role in the horizontal spread of *bla* genes, but the contribution of

82 intracellular transposition is often uncharacterised in population-level studies. Movement

83 of *bla* genes from chromosomal sites to plasmids, or between plasmids, can increase their

84 intercellular transfer potential. Alternatively, transposition from plasmids into chromosomal

85 sites might increase the stability of *bla* genes in new hosts. The insertion sequence IS*Ecp1* is

86 a major driver of intracellular $bla_{CTX-M}$ mobility.[5,6] IS*Ecp1* can mobilise adjacent DNA by

87 recognising alternatives to its right inverted repeat sequence and generating transposition

88    units (TPUs) of various sizes.[5] Because TPUs can carry sequences from adjacent to their

89    previous insertion site, in some cases it is possible to deduce their recent histories by

90    examining their content.

91

92    It is important to understand the diversity and transmission dynamics of both ESBL-EC and

93    *bla* gene-associated MGEs in hospital settings, particularly in intensive care units (ICUs) that

94    host the most vulnerable patients. Although colonisation by antibiotic-resistant *E. coli* has

95    been described as a significant risk for infection in hospitals,[7] genomic surveillance studies

96    have rarely included ESBL-EC that are not derived from clinical specimens.[8] Genomic

97    characterisation of ESBL-EC carried asymptomatically by patients or present in hospital

98    environments might provide insights into the dissemination of ESBL resistance. A recent

99    genomic surveillance study of *Klebsiella pneumoniae* in a Chinese ICU highlighted the utility

100   of considering environmental isolates when assessing hospital populations.[9]

101

102   Here, we have performed a prospective observational study to examine the ESBL-EC

103   population of an ICU in Guangzhou, China. By sampling the entire ICU patient cohort and

104   the ICU environment weekly, and collecting rectal swabs from ICU staff, we have captured a

105   three-month snapshot of ESBL-EC, ESBL-resistance determinants and their associated MGEs.

106   This allowed us to assess the impact of *E. coli* and MGE transmission on the spread and

107   persistence of ESBL resistance in this setting.

108

109   **Materials and Methods**

110   **Ethics**

111    This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of

112    Guangzhou Medical University on May 21$^{st}$, 2018.

113

114    **Study design and sampling regimen**

115    This study was conducted in the Internal Medicine ICU of a tertiary care hospital in

116    Guangzhou, China. Sampling occurred weekly over a 13-week period between July and

117    October 2019. Environmental samples were collected from eight single-bed rooms, a six-bed

118    room, and common areas between rooms. A complete list of environmental sampling sites

119    can be found in Table S1. Oral and rectal swabs were obtained from each patient present in

120    the ward on each weekly sampling occasion.  Staff rectal and coat swabs were collected on

121    three dates over the course of the study.  Swabbing was performed with Copan swabs

122    moistened with Mueller-Hinton broth. Environmental sites were swabbed for 1 minute and

123    transported to the laboratory at room temperature for culturing. Clinical isolates obtained

124    between September and October 2020 were provided by the hospital's clinical laboratory.

125

126    **Bacterial isolation and antibiotic susceptibility testing**

127    Swabs were incubated, shaking, at 37°C in 4 mL of Mueller-Hinton broth until turbidity was

128    observed (usually 16-18 hours, maximum 24 hours). Turbid cultures (50 μL) were spread on

129    CHROMagar ESBL plates and incubated overnight at 37°C. Presumptive *E. coli* colonies were

130    streaked on antibiotic-free Mueller-Hinton agar plates and incubated overnight at 37°C.

131    Single colonies from Mueller-Hinton plates were collected for storage at -80°C, further

132    characterisation and whole-genome sequencing. Species identity was confirmed by MALDI-

133    TOF. Sensitivity to imipenem and meropenem was assessed by broth microdilution

134    according to CLSI guidelines (M100-S26). *E. coli* ATCC 25922 was used as a quality control

135    strain.

136

137    **Plasmid transfer assays**

138    Transfer of the $bla_{CTX-M-55}$-bearing plasmid pDETEC16 was assessed by mating host *E. coli*

139    DETEC-P793 with rifampicin-resistant *E. coli* Ec600. DETEC-P793 and Ec600 overnight

140    cultures (100 μL each) were spread on the same Mueller-Hinton agar plate and incubated at

141    37°C overnight. The resulting lawn was harvested and serially diluted in 0.9% sterile saline.

142    Dilutions were plated on Mueller-Hinton agar containing 20 μg/mL cefepime to select for

143    DETEC-P793, 500 μg/mL rifampicin to select for Ec600, or 20 μg/mL cefepime + 500 μg/mL

144    rifampicin to select for pDETEC16-containing Ec600 transconjugants. Transconjugants were

145    screened for the presence of pDETEC16 and the putative conjugative plasmids pDETEC13,

146    pDETEC14 and pDETEC15 by PCR. Primers and PCR conditions are listed in Table S2.

147

148    **Whole genome sequencing and analysis**

149    Genomic DNA was extracted using a Qiagen minikit (Qiagen, Hilden, Germany) in

150    accordance with the manufacturer's instructions. Whole genome sequencing was

151    performed using both the Illumina HiSeq (Illumina, San Diego, USA) and the Oxford

152    Nanopore GridION (Nanopore, Oxford, UK) platforms (Tianke, Zhejiang, China).

153

154    Illumina sequence reads were trimmed and assembled with Shovill v1.1.0 under default

155    settings with a 10x minimum contig coverage (https://github.com/tseemann/shovill). Read

156    quality was determined with FastQC v0.11.8,1 and assemblies were assessed for

157    contamination and completeness using QUAST v5.0.2, CheckM v1.0.13 and ARIBA v2.14.1

158     with the "Escherichia coli" MLST database. All genomes meeting quality expectations had a

159     total genome size of 4,580,428- 5,537,816 bp; N50 ≥ 65,734; GC content of 50.23 – 50.93 %;

160     genome completeness ≥97.46%; ≤2.52% contamination; ≤251 contigs and complete MLST

161     genes without nucleotide heterogeneity.

162

163     For hybrid assemblies, Nanopore reads were trimmed with Filtlong v0.2.0

164     (https://github.com/rrwick/Filtlong) under default settings targeting approximately 100-fold

165     genome coverage. These were assembled with the trimmed Illumina reads using Unicycler

166     v0.4.8[10] under default settings. For genomes that did not assemble contiguously in this way,

167     Flye v2.7-b1585[11] was used to assemble long reads first. The resulting Nanopore-only

168     assemblies were input into Unicycler along with short reads under default settings or using

169     bold mode where specified (Table S3). Manual approaches were used to complete some

170     assemblies.

171

172     **Genome characterisation**

173     Genomes were initially characterised by using abricate (v0.8.13) to screen with the NCBI

174     AMRFinderPlus and PlasmidFinder databases (both updated 22/09/2021)[12,13]. F-type

175     plasmid replicons were sub-typed using the PubMLST database

176     (https://pubmlst.org/organisms/plasmid-mlst).

177

178     Phylogenetic analysis was undertaken for all isolates together, and separately for each ST

179     with more than three isolates. Reference genomes are listed in Table S4. Reference

180     genomes were annotated with Prokka 1.14.0[14] under default settings. Using Snippy v4.4.5

181     (https://github.com/tseemann/snippy), isolates from the whole dataset and from each ST

182    were aligned against their appropriate reference genome and a core genome alignment was

183    generated. When more than three isolates were represented in each alignment,

184    recombination was removed using gubbins v2.4.0[15] with the Fasttree tree builder[16]. SNP-

185    distances were calculated from resulting core-genome alignments with SNP-dists v0.6.3

186    (https://github.com/tseemann/snp-dists). Phylogenetic trees were constructed with

187    Fasttree v2.1.10 using the nucleotide alignment setting and a general time reversible

188    model[16].

189

190    **Plasmid and translocatable element characterisation**

191    Gene Construction Kit v4.5.1 (Textco Biosoftware, Raleigh, USA) was used to examine and

192    manually annotate plasmid and other mobile DNA sequences.

193

194    **Results**

195    **The intensive care unit hosts a diverse *E. coli* population**

196    Over a three-month period in 2019, 299 samples were collected from ICU patients (59 ESBL-

197    EC-positive; 19.7%), 82 from ICU staff (38 ESBL-EC-positive; 46.3%) and 2967 from the ICU

198    environment (110 ESBL-EC-positive; 3.7%). A total of 131 ESBL-EC isolates were sequenced

199    (Figure 1, Table S5). Sequenced ICU surveillance isolates were derived from patient oral

200    swabs (10 isolates) and rectal swabs (38 isolates), the ICU environment (47 isolates), and

201    from staff rectal swabs (36 isolates). Sinks were the most common sources of environmental

202    isolates (32 of 47 isolates, 68.1%), which were obtained from sink countertops (12 isolates),

203    overflows (9 isolates) drains (8 isolates), taps (2 isolates) or water (1 isolate). The remaining

204    environmental isolates were found on bed unit or equipment surfaces, including those of

205    bed remotes (5 isolates) and bed curtains (1 isolate), a locker (1 isolate), ventilators (2

206    isolates), a nebuliser (1 isolate) and drip stands (2 isolates). One isolate was collected from a

207    door handle, one from a cleaning cart and one from a doctor's coat.  A further 34 ESBL-EC

208    isolates were obtained from clinical samples taken from patients throughout the hospital

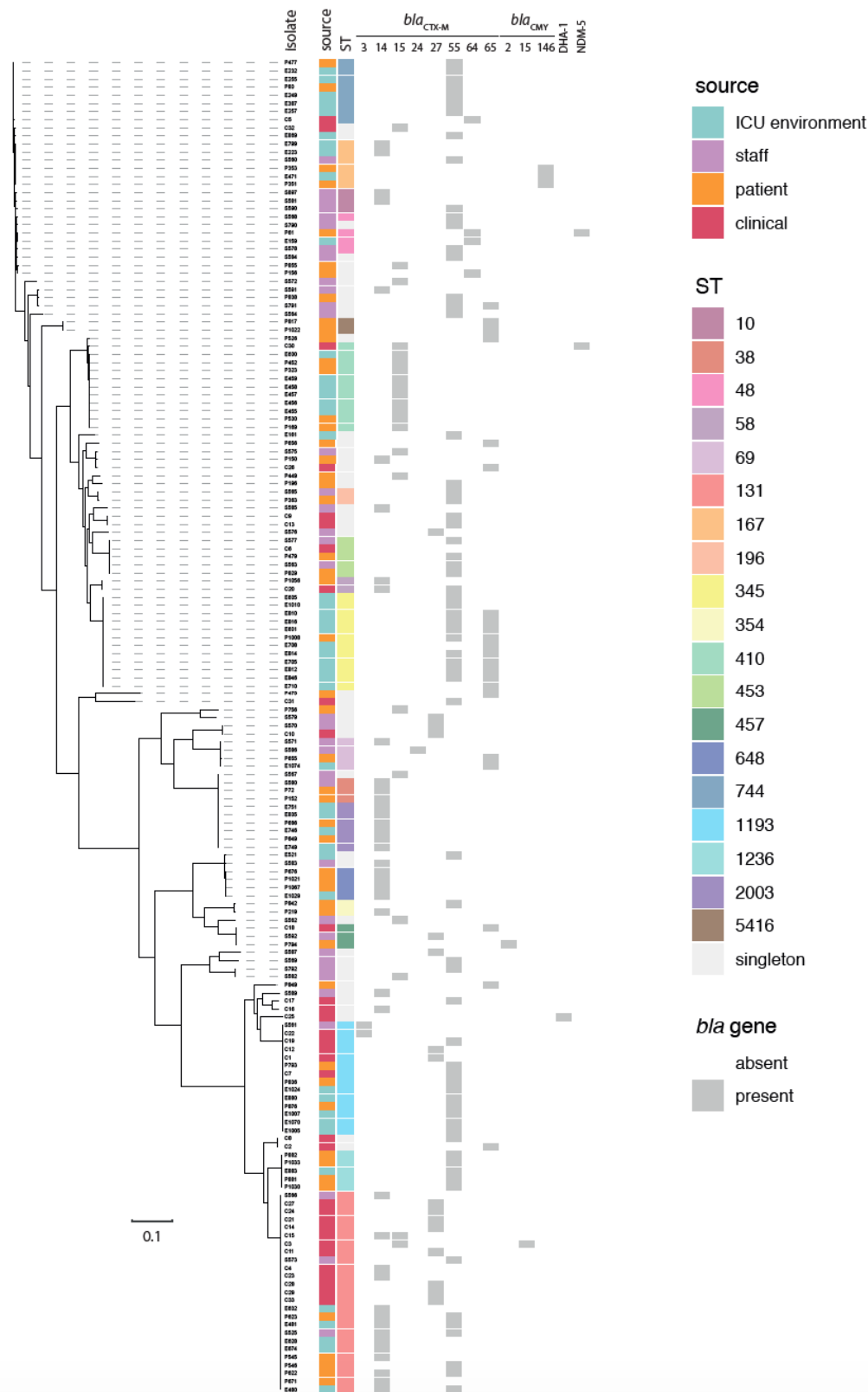209    over a two-month period in 2020.

210

**Figure 1:** ESBL-EC collection phylogeny. Core-gene phylogeny of the ESBL-EC collection assembled in this study. Isolate names are labelled to the right of dashed lines that indicate their positions in the phylogeny. To the right of the phylogeny, sources of isolation, sequence type (ST) designations and the presence or absence of

215     *bla* genes are indicated by colours as outlined in the key. High-resolution figure included with supplementary
216     materials.

217    Multi-locus sequence typing revealed 50 different sequence types (STs) in the ICU

218    surveillance collection (131 isolates) and 17 in the clinical collection (34 isolates). One ICU

219    surveillance isolate and one clinical isolate were novel types, which were submitted to

220    Enterobase and assigned ST12546 and ST12742. Of the 59 STs in the entire collection, 36

221    were only represented by single isolates and 19 were represented by between two and

222    eight isolates. The most prevalent STs in the collection were ST131 (25 isolates), ST1193

223    (14), ST345 (11) and ST410 (11). Eight of the 17 STs in the clinical collection were also

224    present in the ICU surveillance collection, namely ST131, ST1193, ST410, ST744, ST453,

225    ST58, ST393 and ST457.

226

227    **Environmental ESBL-EC isolates were strongly associated with patient carriage**

228    Visualising the distribution of ESBL-EC isolates, patients and bed units over the course of the

229    surveillance study revealed patterns of ST, patient and environmental associations in the

230    ICU (Figure 2). ESBL-EC was isolated from a patient or their bed unit on 60 sampling

231    occasions that involved 30 different patients. On 32 of these occasions, isolates were

232    derived from only the patient; on 15 occasions from only the environment; and on 13

233    occasions from both the patient and their bed unit environment. On nine of the 13

234    occasions when ESBL-EC was isolated from both the patient and their bed unit environment,

235    the environmental and patient isolates were the same ST. Of the 15 occasions on which

236    ESBL-EC was isolated from a bed unit environment but not its resident patient, in nine the

237    environmental isolate's ST was the same as isolates that had been collected from that

238    patient in the week(s) prior. Thus, of 60 sampling occasions where ESBL-EC was isolated

239    from occupied bed units, 50 occasions (83.3%) involved STs obtained directly from

240    occupying patients at the time of sampling or on previous sampling occasions.
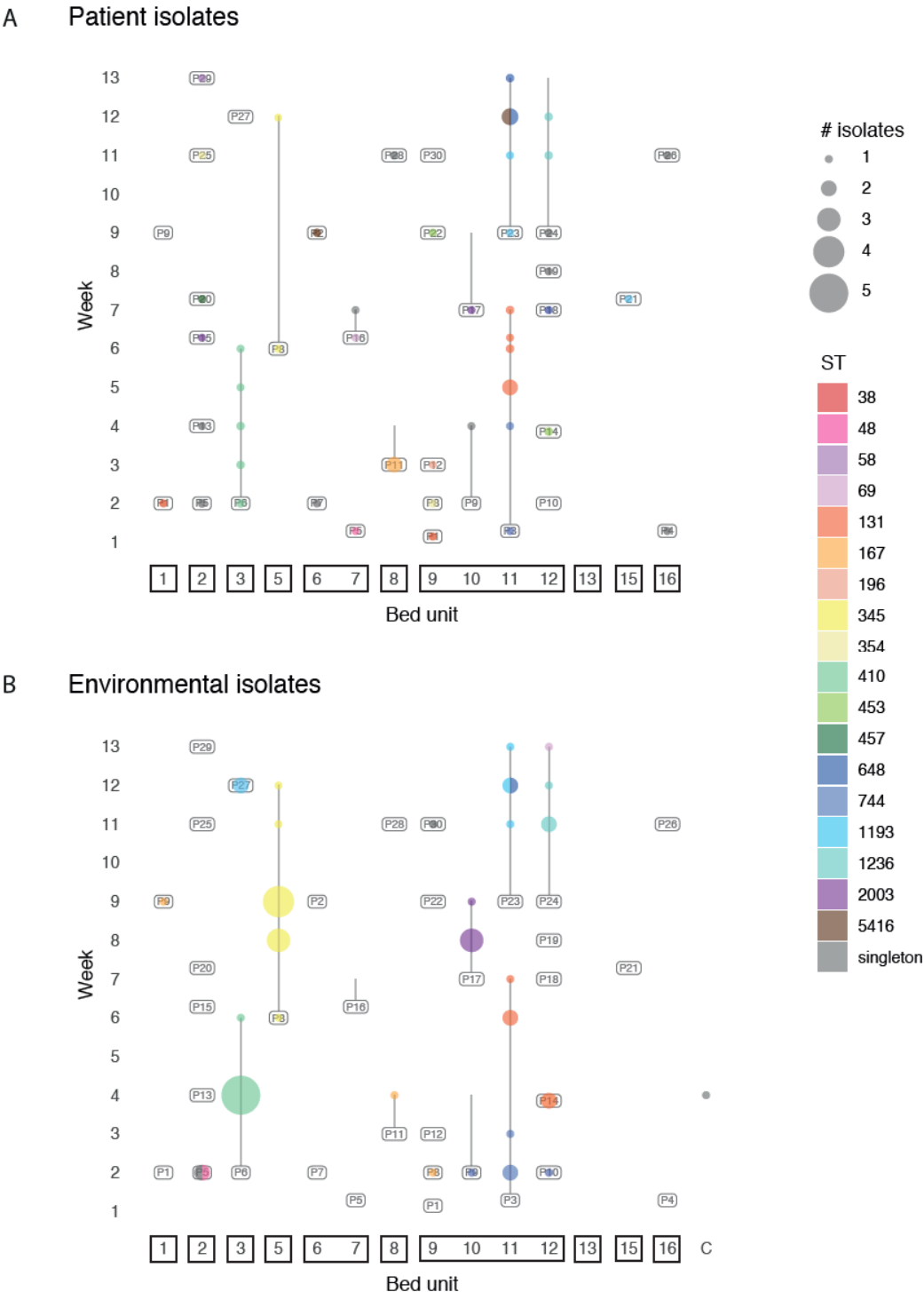
241



**Figure 2:** Distribution of ESBL-EC STs in the ICU surveillance study. Bubble plot showing the distributions of **A)** patient-derived and **B)** ICU environment-derived ESBL-EC isolates over the course of the ICU surveillance study. The locations in which STs were isolated are indicated by coloured bubbles, with the sizes of bubbles indicative of the number of isolates obtained. C = common areas outside bed units.

248    In all 11 cases where a patient and their bed unit were sampled longitudinally, at least one

249    ST was isolated from patient or bed unit on multiple sampling occasions. ESBL-EC isolates

250    associated with a patient and their bed unit were usually a single ST throughout the

251    patient's ICU stay. Only five patients (P3, P8, P16, P23, P24) were associated with carriage of

252    multiple STs, with those different STs isolated on separate sampling occasions (Figure 2A).

253    Two patients (P1, P8) moved between bed units during the study, and yielded ESBL-EC from

254    oral or rectal swabs in both locations. In both cases the same ST was isolated in both

255    locations (Figure 2A).

256

257    **Evidence for strain persistence and dissemination in ICU environments**

258    Across the ICU surveillance and clinical collections, 19 STs were associated with multiple

259    environments, patients or staff members. To determine whether isolates of the same ST

260    were closely related and might be derived from a single introduction to the ICU, we

261    evaluated core-genome SNP (cgSNP) distances as well as plasmid replicon and antibiotic

262    resistance gene content. Where available, whole genome sequences were also compared to

263    confirm the relationships between closely-related isolates.  Distances between isolates of

264    the same ST ranged from 0 to 20,795 cgSNPs (median= 311 SNPs; IQR = 114 - 4536 SNPs;

265    Table S6).   The median maximum cgSNP distance between isolates of the same ST

266    associated with a single patient was 3 cgSNPs (IQR = 1 - 9 cgSNPs), though up to 99 cgSNPs

267    were found between ST345 isolates associated with P8 (Table S7). Cases where closely

268    related isolates of the same ST were present in multiple bed units are outlined below.

269

270    ST744 isolates were obtained from adjacent bed units 10, 11 and 12 between weeks 1 and 4

271    (Figure 2). ST744 first appeared in BU11 in week 1, isolated from a P3 rectal swab. It was

272     then isolated from the BU11 environment in weeks 2 and 3 before it was isolated from

273     another P3 rectal swab in week 4. Isolates from P3 and BU11 differed by a maximum of

274     three cgSNPs. In week 2, ST744 isolates were also obtained from the environments of BU10

275     and BU12, which are in the same room as BU11 (Figure 2B). The BU10 and BU12 isolates

276     differed from the BU11 isolates by 1-2 cgSNPs and 9-13 cgSNPs, respectively. All ST744

277     isolates carried the same ARGs and plasmid replicons.

278

279     The ST744 strain in P3 appears to have been displaced by a ST131 strain over P3's time in

280     the ICU. From week 5 until their discharge from the ICU after sampling in week 7, P3 yielded

281     ST131 isolates from oral and rectal swabs (Figure 2A). ST131 isolates were also obtained

282     from the BU11 environment in weeks 6 and 7 (Figure 2B). The P3 ST131 isolates differed by

283     1-8 cgSNPs from two isolates obtained from equipment in the adjacent BU12 a week earlier.

284     Complete genomes were obtained for DETEC-E480, isolated from the BU12 environment in

285     week 4, and DETEC-P622, isolated from a P3 rectal swab in week 6. Both genomes contain

286     six plasmids, five of which are identical (Figure S1). The sixth plasmid in each genome is an

287     FII-33:N cointegrate that contains multiple antibiotic resistance genes. The FII-33:N plasmid

288     in DETEC-E480, pDETEC56, is 103,838 bp and pDETEC60 in DETEC-P622 is 82,673 bp. The

289     difference in size is accounted for by an IS$26$-mediated deletion event which has removed

290     the *fosA3*, *sul2*, *strAB*, *tet*(A) and *floR* genes from pDETAB60, leaving only *rmtB*, $bla_{TEM}$ and

291     $bla_{CTX-M-55}$ (Figure S1). One ST131 isolate obtained from P3 in week 5, and all ST131 isolates

292     from P3 or BU11 after week 6 did not contain FII-33 or N replicons, or the resistance genes

293     associated with the FII-33:N cointegrate, suggesting that this plasmid had been lost. A

294     further ST131 isolate that differed from those in P3/BU11 by 0-8 cgSNPs and contained the

295     FII-33:N replicons and associated ARGs was isolated from a doctor's coat in week 8.

296

297    After P3 had been discharged, P23 occupied BU11 from week 9 to the end of the study in

298    week 13. Over this period, 11 ST1193 isolates were isolated from P23 and the BU11

299    environment, including from the sink. These isolates differed by 0-8 cgSNPs. In week 12, two

300    ST1193 isolates were obtained from the sink in BU3 (Figure 2B). The BU3 sink isolates

301    differed from the P23/BU11 ST1193 isolates by 0-7 cgSNPs, and all BU11/BU3 isolates

302    carried the same ARGs and plasmid replicons. Complete genomes were obtained for the

303    ST1193 isolates DETEC-P836 from a P23 rectal swab, DETEC-E1005 from the BU3 sink and

304    DETEC-E1070 from the BU11 sink. All three genomes contain the identical plasmids pDETEC3

305    and pDETEC4.

306

307    Other examples of closely related isolates from different patients or ICU environments

308    include an ST5416 isolated in week 12 from P23 from an oral swab. This ST had only

309    previously been isolated from P2 in bed unit 6, in week 9, which was the same week P23

310    was admitted to the ICU. The ST5416 isolates differed by 1 cgSNP and contained the same

311    ARGs and plasmid replicons. ST167 isolates with 0 cgSNPs were found seven weeks apart,

312    from the bed curtain of BU9 in week 2 and from the sink overflow of BU1 in week 9,

313    associated with P8 and P9, respectively (Figure 2B). P8 had occupied BU9 in week 2, at

314    which point they were adjacent to P9, who was in BU10 from week 2 to week 4. After a

315    four-week absence from the ICU, P9 was in BU1 when ST167 was isolated from its sink

316    overflow. ST174 isolates that differed by 1 cgSNP were obtained from rectal swabs from two

317    different staff members, but they carried different $bla_{CTX-M}$ genes. ST393 isolates from a staff

318    rectal swab and a clinical specimen from 2020 carried $bla_{CTX-M-27}$ had identical core genomes

319    (0 cgSNPs). These appeared to represent the only ESBL-EC strain found in both the clinical

320    and ICU surveillance collections.

321

322    **Diverse ESBL resistance determinants were found in diverse genetic contexts**

323    CTX-M-type β-lactamases were the dominant ESBL resistance determinants in this

324    collection, with one or more $bla_{CTX-M}$ genes found in 159 of the 165 isolates (96.4%). Most

325    isolates (143/159; 89.9%) contained a single $bla_{CTX-M}$ gene, while 16 contained two different

326    $bla_{CTX-M}$ genes (Figure 1). The $bla_{CTX-M-55}$ gene was the most common in the collection (67

327    isolates; 27 STs), followed by $bla_{CTX-M-14}$ (41 isolates; 15 STs), $bla_{CTX-M-15}$ (23 isolates; 7 STs),

328    $bla_{CTX-M-65}$ (22 isolates; 11 STs), $bla_{CTX-M-27}$ (15 isolates; 7 STs), $bla_{CTX-M-3}$ (2 isolates; both

329    ST1193) and $bla_{CTX-M-24}$ (1 isolate; ST69). Amongst the isolates that carried two $bla_{CTX-M}$

330    genes, nine had $bla_{CTX-M-55}$ with $bla_{CTX-M-65}$, six had $bla_{CTX-M-55}$ with $bla_{CTX-M-14}$ and one had

331    $bla_{CTX-M-15}$ with $bla_{CTX-M-14}$. Of the six that lacked $bla_{CTX-M}$ genes, three ST167 isolates carried

332    $bla_{CMY-146}$, a ST457 isolate carried $bla_{CMY-2}$, a ST706 isolate carried $bla_{DHA-1}$ and a ST453 isolate

333    carried only $bla_{TEM}$ (Figure 1).

334

335    We determined the context of $bla_{CTX-M}$ genes in 93 of the 165 isolates in the collection, by

336    examining complete genomes (23 isolates) or $bla_{CTX-M}$-containing contigs in draft genomes

337    (70 isolates). In the remaining cases $bla_{CTX-M}$ genes were found in contigs that only included

338    mobile element sequences and therefore did not contain sufficient information to reliably

339    determine their locations. Of the 93 instances where the locations of $bla_{CTX-M}$ genes were

340    determined, 44 were in chromosomes and 50 in plasmids (one isolate carried copies of

341    $bla_{CTX-M-55}$ n its chromosome and in a plasmid). In 55 cases $bla_{CTX-M}$ genes were located in

342    complete IS*Ecp1* TPUs for which boundary sequences could be determined. The sizes of

343    these TPUs ranged from 2,841 bp to 18,201 bp (Table S5).

344

345    The 55 complete IS*Ecp1-bla*$_{CTX-M}$ TPUs were inserted in 18 different positions in

346    chromosomes and seven in plasmids (Table S5). All complete TPUs were flanked by 5 bp

347    target site duplications (TSDs). Three TPU-insertion position combinations were seen in

348    multiple STs. A 2,845 bp chromosomal unit was flanked by the TSD TGTTT in position in five

349    ST1236 isolates, and one isolate each of ST1485 and 3941. The other combinations found in

350    multiple STs were associated with I-complex plasmids: a 2,971 bp unit in an I1 plasmid was

351    in five STs and a 3,060 bp unit in a Z plasmid was in three STs. This suggested that *bla*$_{CTX-M}$-

352    bearing I1 and Z-type plasmid lineages might be circulating in this *E. coli* population.

353

354    **I-complex plasmid lineages found in multiple STs**

355    To investigate the possibility that the same I1 and Z plasmid lineages were present in

356    multiple STs in this *E. coli* population, we compared complete plasmid sequences to one

357    another and to contigs from draft genomes that represent incomplete plasmid sequences.

358

359    I1 plasmids containing a 2,971 bp IS*Ecp1-bla*$_{CTX-M-55}$ TPU flanked by the TSD TACTT were

360    found in six isolates in this collection: one ST1011 and two ST1193 isolates from clinical

361    specimens, and one isolate each of ST93, ST167 and ST196 from ICU staff rectal swabs. The

362    backbones of these plasmids were typical representatives of the I1 type (Figure 3A),

363    containing shufflons and complete transfer regions like those of the reference plasmid

364    R64.[17] Based on the presence of two recombinant patches in their backbones, the I1

365    plasmids in this collection were divided into two sub-lineages, represented by pDETEC69

366    and pDETEC73 in Figure 3A. The plasmids from ST93 and ST167 isolates belonged to the

367    pDETEC69 sub-lineage, while those from ST196, ST1011 and ST1193 isolates belonged to the

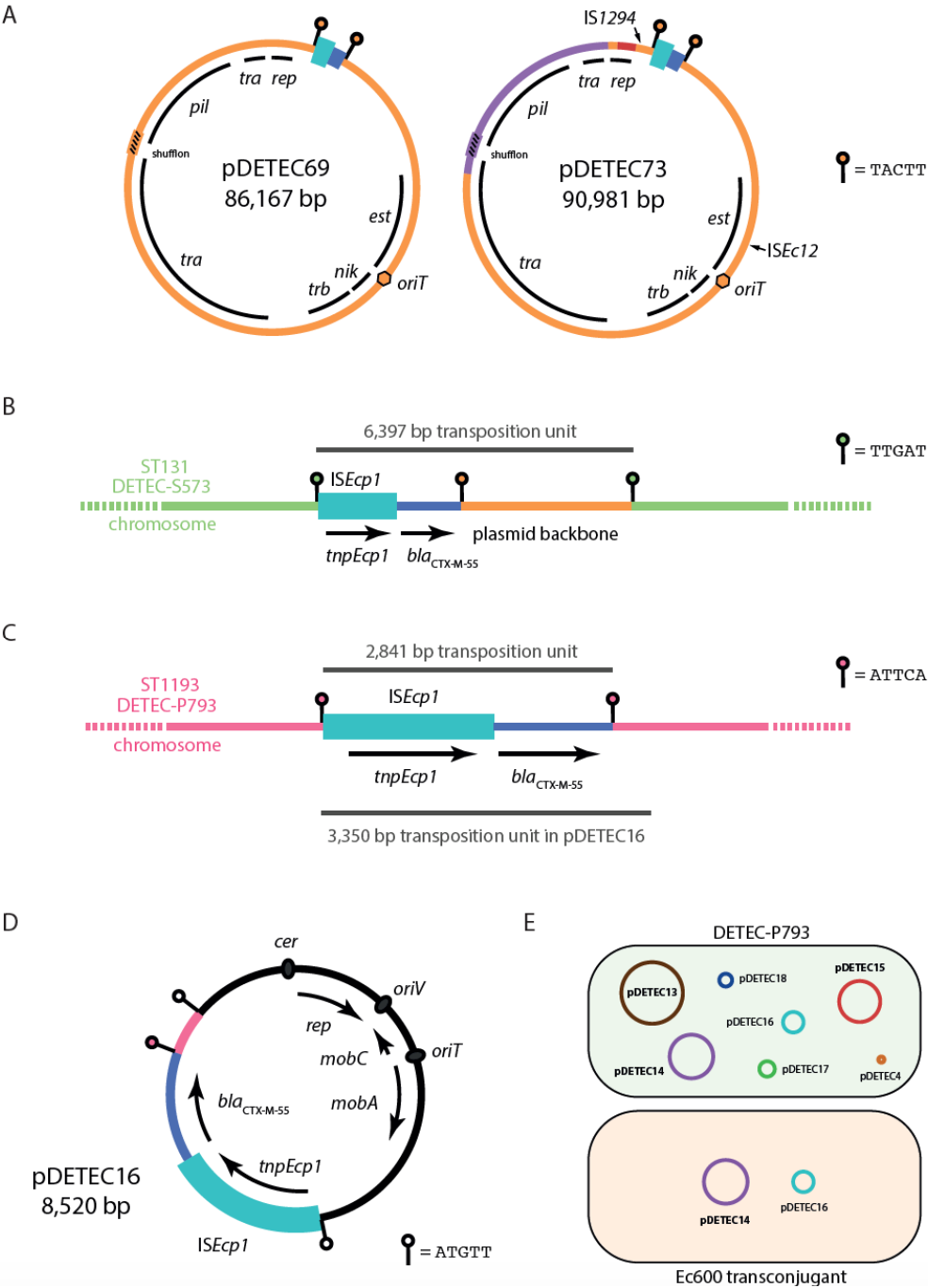368    pDETEC73 sub-lineage.

369



370
371

372   **Figure 3:** Plasmids, transposition units and $bla_{CTX-M}$ movement. **A)** Circular maps of the I1 plasmids pDETEC69
373   and pDETEC73. The extents of replication (*rep*), transfer (*tra, trb*), thin pilus biogenesis (*pil*) and establishment
374   (*est*) regions are shown. IS*Ecp1-bla*$_{CTX-M}$ TPUs are shown as cyan/blue boxes flanked by lollipops that indicate
375   the position and sequence of target site duplications. Purple and maroon segments in pDETEC73 represent
376   recombinant sequences. **B)** IS*Ecp1-bla*$_{CTX-M-55}$ TPU in the chromosome of ST131 isolate DETEC-S573. **C)** IS*Ecp1-*
377   *bla*$_{CTX-M-55}$ TPU in the chromosome of ST1193 isolate DETEC-P793. **D)** Small $bla_{CTX-M-55}$-bearing plasmid
378   pDETEC16. **E)** Co-transfer of pDETEC16 and pDETEC14. Shaded cells represent DETEC-P793 and a
379   transconjugant derived from mating DETEC-P793 with *E. coli* Ec600. The plasmids in each host are shown as
380   labelled circles. Parts A to D of this figure are drawn to different scales, though IS*Ecp1* (1,656 bp) is shown in
381   each, and the sizes of TPUs in parts B and C are indicated.
382
383   Z plasmids containing a 3,050 bp IS*Ecp1-bla*$_{CTX-M-14}$ TPU flanked by the TSD GCGGA were

384   found in four isolates in this collection: a ST131 isolate from a clinical specimen, a ST58

385   isolate from an ICU patient rectal swab, and ST95 and ST131 isolates from ICU staff rectal

386   swabs. Similar to the situation seen amongst I1 plasmids, the Z plasmids could be divided

387   into sub-lineages on the basis of backbone recombination patches. Plasmids from the

388   patient ST58 (pDETEC82) and staff ST131 (pDETEC79) isolates belonged to the same sub-

389   lineage. Apart from rearrangements in the shufflon region (which was also interrupted by

390   IS*1* in pDETEC82), pDETEC79 and pDETEC82 were almost identical (99.98% nucleotide

391   identity across 85,765 bp compared).

392

393   Both of the signature TPU-backbone junction sequences from the I1 and Z plasmids

394   described above were found in multiple plasmids in GenBank, indicating that these lineages

395   are present in wider enterobacterial populations. Plasmids bearing the I1 plasmid TACTT-

396   flanked IS*Ecp1-bla*$_{CTX-M-55}$ insertion (n = 25) have been seen in *E. coli*, *Shigella sonnei*,

397   *Salmonella* Typhimurium, *Klebsiella pneumoniae* and *Enterobacter hormachei* that were

398   isolated from human faeces, clinical isolates, animals and wastewater in China (n = 18),

399   Japan (n = 3), Kazakhstan, Belgium, Switzerland and the UK (n = 1 each) (Table S8). Plasmids

400   containing the Z-plasmid GCGGA-flanked IS*Ecp1-bla*$_{CTX-M-14}$ insertion (n = 44) have been

401    carried by *E. coli*, *K. pneumoniae*, *Salmonella* and *Shigella* isolated from multiple countries in

402    Asia and Europe, as well as in Australia and the USA (Table S9).

403

404    **Evidence linking chromosomal *bla*$_{CTX-M}$ genes to specific plasmid lineages**

405    To investigate the dynamics of their inter-host and inter-molecular transmission, we

406    examined the contents of complete chromosomal IS*Ecp1*-*bla*$_{CTX-M-55}$ TPUs. In six cases we

407    were able to definitively identify the plasmid lineages that chromosomal insertions were

408    derived from. In the ST131 isolate DETEC-S573 from an ICU staff rectal swab, *bla*$_{CTX-M-55}$ is

409    located in a 6,397 bp IS*Ecp1* TPU inserted in the chromosome and flanked by the TSD TTGAT

410    (Figure 3B). The 6,397 bp TPU includes 3,426 bp of I1 plasmid backbone from immediately

411    adjacent to the 2,971 bp TPU described above, including one copy of the associated TSD

412    sequence TACTT. Thus, we conclude that the 6,397 bp TPU in this ST131 chromosome is

413    derived from the I1 plasmid lineage present in multiple STs in this ESBL-EC population

414    (Figure 3B). As DETEC-S573 does not contain an I1 plasmid, the plasmid must have been lost

415    after delivering the *bla*$_{CTX-M-55}$ gene. Similarly, we determined that a HI2 plasmid lineage

416    (GenBank accession MT773678) was the source of the 18,201 bp chromosomal IS*Ecp1*-

417    *bla*$_{CTX-M-64}$ TPU in two ST48 isolates, a second HI2 plasmid lineage (AP023198)  the source of

418    the 3,050 bp chromosomal IS*Ecp1*-*bla*$_{CTX-M-55}$ TPU in a ST12742 isolate, an I2 plasmid

419    (LR890295) the source of the 5,800 bp chromosomal IS*Ecp1*-*bla*$_{CTX-M-55}$ TPU in a ST617

420    isolate, and an I-complex plasmid lineage not represented in this collection or in GenBank

421    was the source of the 3,445 bp chromosomal IS*Ecp1*-*bla*$_{CTX-M-14}$ TPU in a ST345 isolate (Table

422    S5). The FII-2 plasmid lineage represented by pHK01 (HM355591), which is present in clinical

423    ST12 isolate DETEC-C16 from this collection, was the source of the 4,477 bp chromosomal

424    IS*Ecp1*-*bla*$_{CTX-M-14}$ TPU in two ICU patient ST38 isolates (Table S5).

425

426 **Chromosome-to-plasmid transposition of $bla_{CTX-M-55}$ in ST1193**

427 The complete genome of ST1193 patient rectal isolate DETEC-P793 contained two copies of

428 $bla_{CTX-M-55}$, one in the chromosome and one in a small plasmid. The chromosomal copy is in a

429 2,841 IS$Ecp1$ TPU (Figure 3C). The second copy is in the 8,520 bp ColE2-like plasmid

430 pDETEC16 (Figure 3D). The IS$Ecp1$-$bla_{CTX-M-55}$ TPU in pDETEC16 is 3,350 bp and flanked by the

431 5 bp target site duplication ATGTT (Figure 3D). The final 509 bp of the TPU are identical to

432 the sequence adjacent to the DETEC-P793 chromosomal TPU (Figure 3D). This indicates that

433 the TPU in pDETEC16 was acquired from its host's chromosome. pDETEC16 has a ColE2-like

434 backbone that contains a putative origin-of-tranfer ($oriT$) and MOB$_{Q4}$-type mobilisation

435 determinants (Figure 3D).

436

437 Three of the seven plasmids carried by DETEC-P793 contain complete transfer regions

438 (Figure 3E). We mated DETEC-P793 with $E. coli$ Ec600 in order to determine whether any of

439 the large plasmids in DETEC-P793 could mobilise pDETEC16. Transconjugants were obtained

440 at a mean frequency of 8.55 x $10^{-6}$ per donor. Five transconjugants were screened for the

441 presence of pDETEC16 and all three putative conjugative plasmids by PCR. The I1 plasmid

442 pDETEC14 was detected along with pDETEC16 in all transconjugants, while pDETEC13 and

443 pDETEC15 were not detected in any (Figure 3E). This demonstrated that pDETEC14 had

444 mobilised pDETEC16 in the laboratory. Mobilisation of pDETEC16 by an I1 plasmid is

445 consistent with previous studies that have shown that MOB$_{Q4}$-type plasmids can be

446 mobilised by I-complex plasmids.[18]

447

448 **Carbapenem resistance associated with IS$1$-mediated amplification of $bla_{CMY-167}$**

449    All isolates were tested for susceptibility to meropenem, and just four exhibited resistance.

450    Carbapenem resistance in the ST410 clinical isolate DETEC-C6 and the ST48 patient rectal

451    swab isolate DETEC-P61 could be explained by the presence of the $bla_{NDM-5}$ metallo-ß-

452    lactamase gene. The two remaining meropenem-resistant isolates were ST167 and

453    contained $bla_{CMY-167}$, which is not expected to confer resistance to carbapenems. DETEC-

454    P351 was isolated from a P11 rectal swab in week 3 and DETEC-E471 from P11's bed unit

455    environment a week later (Figure 2). The complete genome of DETEC-P351 contains seven

456    copies of $bla_{CMY-167}$. Six copies are in the 77,960 bp I1 plasmid pDETEC6, and the seventh is in

457    the chromosome (Figure 4A). The copies of $bla_{CMY-167}$ in pDETEC6 are interspersed with

458    copies of IS$1$, in a configuration that resembles structures produced by IS$26$.[19] Consistent

459    with amplification of $bla_{CMY-167}$ by IS$1$ in the I1 plasmid context, we found a putatively

460    ancestral I1 plasmid in GenBank (pECY56; accession KU043116) that contains just a single

461    copy of $bla_{CMY}$, with flanking sequences identical to those in pDETEC6 (Figure 4B).

462

463    The chromosomal $bla_{CMY-167}$ gene in DETEC-P351 lies between two copies of IS$1$ in what

464    appears to be a 4,266 bp compound transposon flanked by the 9 bp TSD GGACCTGCC

465    (Figure 4A). The 2,730 bp passenger sequence between the copies of IS$1$ is identical to the

466    amplified segment in pDETEC6. The chromosomal copy of $bla_{CMY-167}$ is therefore likely to
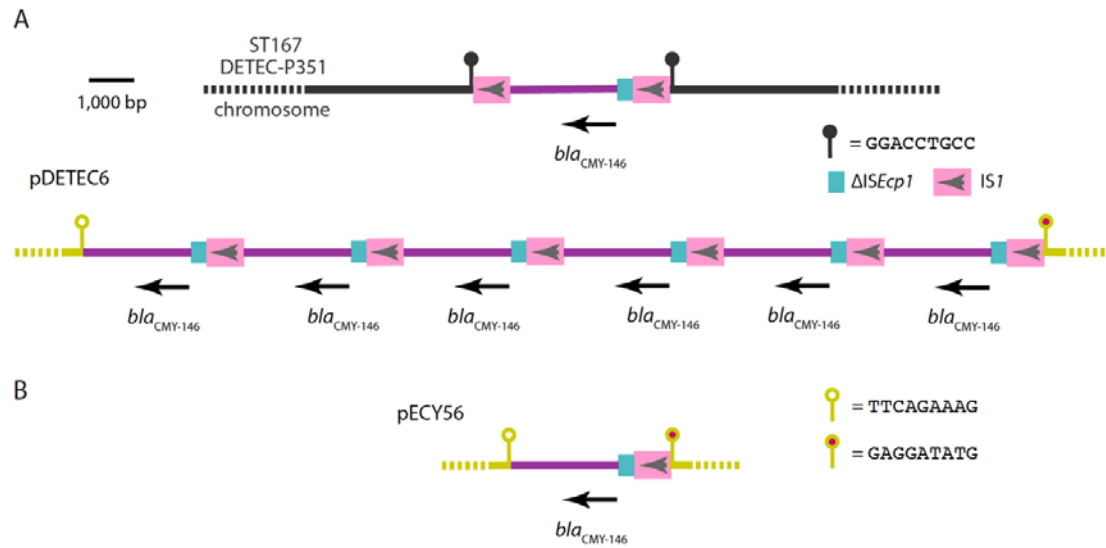
467    have been acquired from pDETEC6.

468

469
470 **Figure 4:** Amplification of $bla_{CMY-146}$ in *E. coli* ST167. Scaled diagrams showing **A)** Contexts of $bla_{CMY-146}$ in DETEC-
471 P351, and **B)** The context of $bla_{CMY}$ in pECY56. IS*1* are shown as pink boxes with arrows indicating the
472 orientation of their transposase genes. Fragments of IS*Ecp1* are shown as cyan boxes, and the amplified
473 sequence containing $bla_{CMY}$ as purple lines. The DETEC-P351 chromosome is shown as a black line, and the
474 pDETEC6/pECY56 backbone as a staggered grey line. Colour-filled lollipops indicate the positions of the
475 sequences shown.
476

477    **Internationally-distributed multi-drug resistance plasmid lineages in the ICU**

478    We have generated 91 complete plasmid sequences as part of this study (Table S10). These

479    represent a diverse spectrum of plasmid types, including commonly-described large

480    plasmids, phage-plasmids, and small plasmids that utilise rolling-circle replication or theta

481    replication with RNA ($\theta$-RNA) or protein ($\theta$-Rep) initiators. Forty of the complete plasmids

482    contain one or more antibiotic resistance genes. Most ARG-containing plasmids were F-

483    types (n=24) or I-complex (n=10), with the remainder X-types (n=2), a phage-plasmid, a H-

484    type plasmid, a $\theta$-RNA plasmid and a $\theta$-Rep plasmid (n=1 each).

485

486    Amongst the F-type plasmids we found examples of well-characterised internationally-

487    distributed lineages. Four complete plasmids contained FII-33 replicons, and when we

488    examined draft genomes we found FII-33 replicons in a further 22 isolates. We have recently

489    described the diversity and evolution of the FII-33 plasmid lineage, which is endemic in

490    China, internationally-disseminated and strongly associated with multi-drug resistance in *E.*

491    *coli* and *K. pneumoniae*.[20] Five complete plasmids were members of F-type ColV/ColBM

492    lineages that carry colicin and virulence genes, and a further 27 draft genomes contain all or

493    part of the *cvaC* colicin V gene. The virulence-associated genes in ColV/ColBM plasmids

494    include those for siderophores such as aerobactin and salmochelin, which are thought to

495    contribute to extra-intestinal virulence in *E. coli*.[21,22] Acquisition of these plasmids has

496    played an important role in the evolution of some pathogenic *E. coli* lineages, and they have

497    been associated with pandemic lineages such as ST131, ST95 and ST58.[23,24] ColV and ColBM

498    plasmid lineages are known to have acquired antibiotic resistance determinants,[24] and all

499    five complete examples in this collection contained multiple resistance genes in complex

500    resistance regions.

501

## Discussion

503    This study has provided a high-resolution three-month snapshot of an ICU's ESBL-resistant

504    *E. coli*. The population was diverse, with strains carried by staff largely distinct from those

505    found in patients and the ICU environment. ESBL resistance determinants were also diverse,

506    and although $bla_{CTX-M-55}$ and $bla_{CTX-M-14}$ dominated, they were found in various contexts in

507    plasmids and chromosomes. Some $bla_{CTX-M}$-bearing plasmid lineages were found across the

508    disparate *E. coli* populations, or were shown to have introduced $bla_{CTX-M}$ genes that

509    transposed into host chromosomes as passengers in IS*Ecp1* TPUs. Our close examination of

510    IS*Ecp1* TPUs also allowed us to detect the movement of $bla_{CTX-M-55}$ from a chromosomal site

511    to a mobilisable plasmid in a ST1193 strain (Figure 3C-E).

512

513    There was a strong relationship between isolates found in ICU patients and those found in

514    their bed unit environments. However, we observed limited strain persistence in the ICU

515    environment. Although instances of highly-similar isolates being found in multiple bed unit

516    environments were rare, we observed more involving units in the six-bed room (BU9-12;

517    ST744, ST131 and ST1193) than we did other rooms (Figure 2). This suggests that ESBL-EC

518    transmission is more likely to occur in multi-bed ICU rooms. Of the three instances where

519    highly-similar isolates were found in bed units in different rooms, two (involving ST1193 and

520    ST167) were associated with sinks. Hospital sinks have been shown in other studies to be

521    important reservoirs of antibiotic-resistant pathogens,[25,26] and to contribute to transmission

522    via plumbing in model systems.[27]

523

524     Although there was little crossover at strain level between the ICU and clinical collections,

525     some $bla_{CTX-M}$-bearing plasmid lineages were represented in both, as well as in multiple STs

526     within the ICU surveillance collection. I-complex plasmids (I1 and Z types) were particularly

527     prominent here. The association of $bla_{CTX-M}$ genes with I-complex plasmids has been noted

528     internationally, and the existence of multiple internationally-disseminated lineages [28]

529     suggests that the confluence of these elements has proven successful on many occasions.

530     However, where and under which conditions these and other plasmids are transferring in

531     bacterial populations remain open questions. We did not find evidence here for horizontal

532     transfer of plasmids in the ICU, though our examination of only a single ESBL-EC colony per

533     sample precluded this.

534

535     The diversity of the ICU ESBL-EC population, and its strong association with patient or staff

536     carriage, appears to suggest that new ESBL-EC strains are introduced to the ICU regularly.

537     The 46.3% ESBL-EC carriage rate observed in staff here is indicative of a high community

538     carriage rate, as the ICU staff are healthy adults residing in Guangzhou. This highlights the

539     importance of genomic studies targeting community commensal *E. coli* populations,[29] which

540     might reveal links to the strains and plasmids that are ultimately associated with hospital

541     infections.

542

543     A concerning finding here was the presence of multiple copies of $bla_{CMY-146}$ in a carbapenem-

544     resistant ST167 strain that lacked carbapenemase genes (Figure 4). This appears to be

545     another example where IS-mediated amplification of a β-lactam[30–32] or aminoglycoside[33]

546     resistance gene has yielded an unexpected phenotype. In previous cases IS*26* has been

547     involved in gene amplification, but here IS*1* was implicated. As IS*1* is not part of the IS*26*

548     family of elements, for which study of transposition mechanisms has provided an

549     explanation for observed structures,[34] similar molecular examinations of IS$1$ transposition

550     are required. More generally, the modulation of clinically-relevant β-lactam resistance

551     phenotypes by IS-mediated gene duplications requires further investigation.

552

553     **Conclusions**

554     The patients, staff and environment of this ICU hosted a diverse ESBL-EC population over

555     our three month-study period. Our data suggest that strains are being introduced to the ICU

556     regularly, likely in association with patients, but that these strains do not persist for

557     extensive periods in ICU environments. Plasmid and IS*Ecp1*-mediated transmission of $bla_{CTX-}$

558     $_M$ genes play major roles in the ongoing spread of ESBL resistance in *E. coli* populations that

559     can enter hospitals.

560

561     **Funding information**

565

566     **Conflicts of interest**

567     The authors declare that there are no conflicts of interest.

568

569

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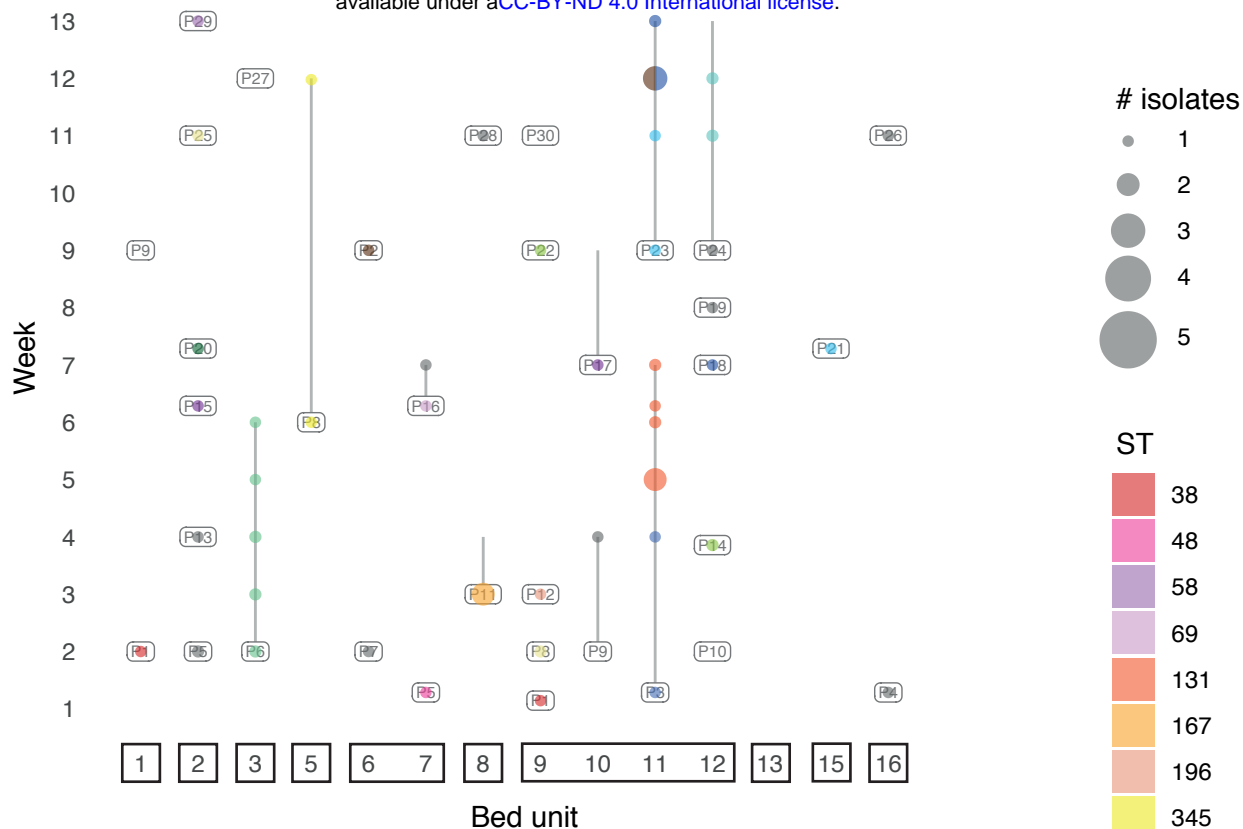
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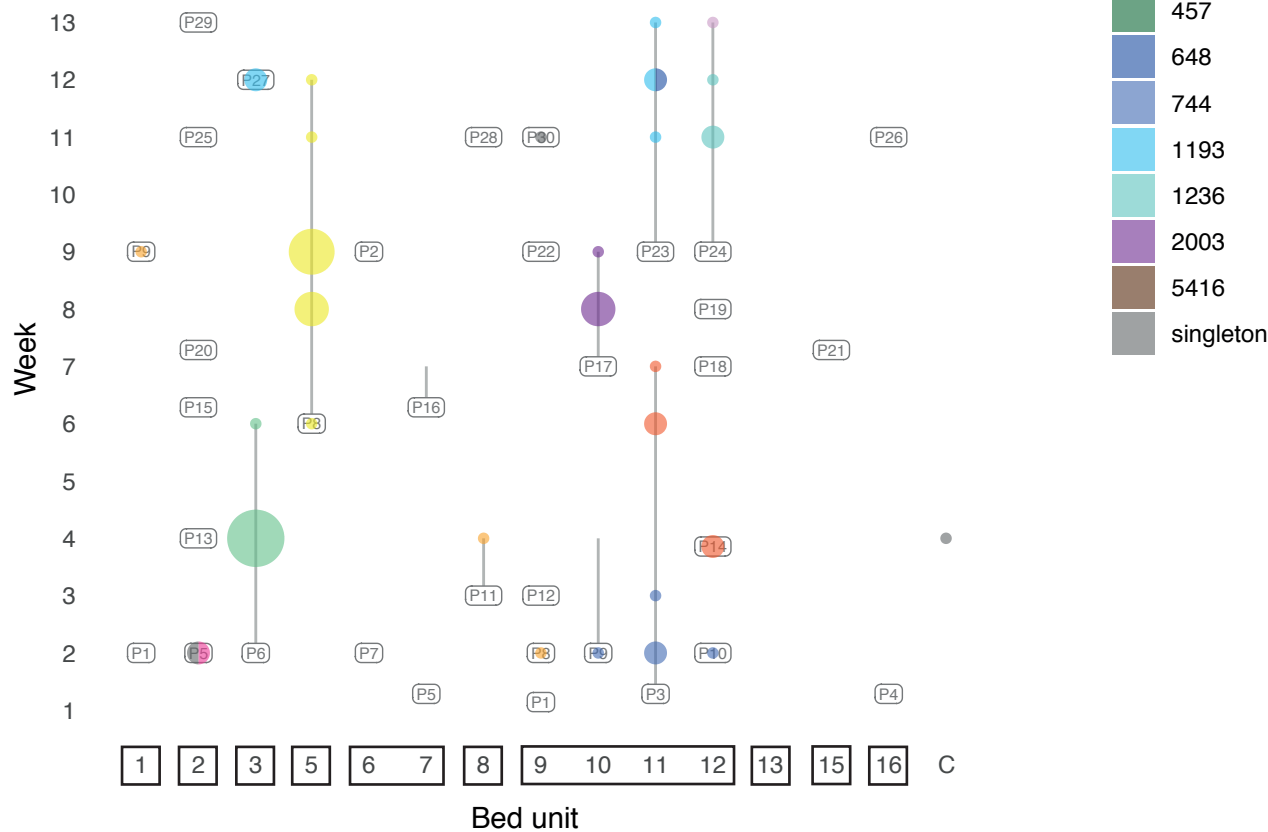658    single family? *Microbial genomics* 2019; **5**.

659

A  Patient isolates

B  Environmental isolates

A

ST167
DETEC-P351

*bla*<sub>CMY-146</sub>

= GGACCTGCC

ΔIS*Ecp1*    IS*1*

pDETEC6

*bla*<sub>CMY-146</sub>    *bla*<sub>CMY-146</sub>    *bla*<sub>CMY-146</sub>    *bla*<sub>CMY-146</sub>    *bla*<sub>CMY-146</sub>    *bla*<sub>CMY-146</sub>

B

pECY56

= TTCAGAAAG

= GAGGATATG

*bla*<sub>CMY-146</sub>