# Piperacillin/tazobactam resistant, cephalosporin susceptible *Escherichia coli* bloodstream infections are driven by multiple acquisition of resistance across diverse sequence types

Thomas Edwards[1, 2 *], Eva Heinz[3,4,5], Jon van Aartsen[6], Alex Howard[6], Paul Roberts[6, 7], Caroline Corless[6], Alice J. Fraser[1, 2], Christopher T. Williams[1, 2], Issra Bulgasim[1, 2], Luis E. Cuevas[3], Christopher M. Parry [4, 8], Adam P. Roberts[1, 2], Emily R. Adams[1, 2], Jenifer Mason[6] and Alasdair T. M. Hubbard[1, 2, 9 *]

[1]Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA

[2]Centre for Drug and Diagnostics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA

[3]Department of Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA

[4]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA

[5]Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, CB10 1SA

[6]Liverpool University Hospital Foundation Trust, Prescot street, Liverpool, L7 8XP

[7]Faculty of Science and Engineering, University of Wolverhampton, Wulfruna Street, Wolverhampton WV1 1LY

[8]Alder Hey Children's NHS Foundation Trust, Eaton Road, Liverpool, L12 2AP

[9]Department of Biosciences, School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS

[*]Corresponding authors:

Dr. Thomas Edwards; email: thomas.edwards@lstmed.ac.uk, telephone: 0151 705 3308

Dr. Alasdair Hubbard; email: alasdair.hubbard@ntu.ac.uk, telephone: 0115 941 8418

**Abstract**

Resistance to piperacillin/tazobactam (TZP) in *Escherichia coli* has predominantly been associated with mechanisms that confer resistance to third generation cephalosporins. Recent reports have identified *E. coli* strains with phenotypic resistance to piperacillin/tazobactam but susceptibility to third generation cephalosporins (TZP-R/3GC-S). In this study we sought to determine the genetic diversity of this phenotype in *E. coli* (*n* = 58) isolated between 2014-2017 at a single tertiary hospital in Liverpool, UK, as well as the associated resistance mechanisms. We compare our findings to a UK-wide collection of invasive *E. coli* isolates (*n* = 1509) with publicly available phenotypic and genotypic data. These data sets included the TZP-R/3GC-S phenotype (*n* = 68), a piperacillin/tazobactam and third generation cephalosporin-susceptible (TZP-S/3GC-S, *n* = 1271) phenotypes. The TZP-R/3GC-S phenotype was displayed in a broad range of sequence types which was mirrored in the same phenotype from the UK-wide collection, and the overall diversity of invasive *E. coli* isolates. The TZP-R/3GC-S isolates contained a diverse range of plasmids, indicating multiple acquisition events of TZP resistance mechanisms rather than clonal expansion of a particular plasmid or sequence type. The putative resistance mechanisms were equally diverse, including hyperproduction of TEM-1, either via strong promoters or gene amplification, carriage of inhibitor resistant β-lactamases, and an S133G $bla_{\text{CTX-M-15}}$ mutation detected for the first time in clinical isolates. Several of these mechanisms were present at a lower abundance in the TZP-S/3GC-S isolates from the UK-wide collection, but without the associated phenotypic resistance to TZP. Our findings highlight the complexity of this cryptic phenotype and the need for continued phenotypic monitoring, as well as further investigation to improve detection and prediction of the TZP-R/3GC-S phenotype from genomic data.

45 **Introduction**

46 *Escherichia coli* is the most common cause of bacterial blood stream infections globally (1), accounting for 27%
47 of all bacteraemic episodes, with a case fatality rate of 12% (2), and causing 78.8 blood stream infections per
48 100,000 people in the UK in 2014 (3). Antimicrobial resistance (AMR) in *E. coli* is increasingly prevalent (4-6)
49 and extended spectrum β-lactamase (ESBL) production, mediating resistance to third generation
50 cephalosporins (3GCs) and other β-lactam antibiotics (7), is of particular concern. ESBLs were recorded in
51 approximately 11% of *E. coli* isolated from blood stream infections in the UK in 2018 (8).

52 One strategy to provide therapeutic options for antimicrobial resistant infections has been the combined use of
53 β-lactamase inhibitors with β-lactam antibiotics to block the activity of β-lactamase enzymes, rendering the
54 bacteria *de facto* susceptible (9). The inhibitor tazobactam, which inhibits class A β-lactamases and includes
55 most ESBL enzymes, is commonly utilised in combination with the penicillin class antibiotic piperacillin (10).
56 Tazobactam is a "suicide inhibitor", as it irreversibly binds to β-lactamases, inactivating the enzyme (11).
57 Piperacillin/tazobactam (TZP) has broad spectrum activity against Gram-negative and -positive bacteria (12), is
58 well tolerated (13), available for paediatric use, and utilised in the UK as a first line empirical agent for serious
59 infections, including pneumonia and intra-abdominal infections (14). Its broad spectrum makes it an important
60 agent for reducing the usage of carbapenem drugs, which are globally important last line antibiotics. Limiting
61 carbapenem use is a critical element of antimicrobial stewardship and essential for preventing the spread of
62 resistance (15). Treatment options for carbapenem resistant bacteria are often limited to poorly tolerated
63 drugs (e.g. colistin or tigecycline) (16). Whilst TZP does possess in-vitro activity against ESBLs, the MERINO trial
64 did not demonstrate non-inferiority of TZP to meropenem in treating patients with ESBL *E. coli* and *K.*
65 *pneumoniae* blood stream infections (17). Carbapenems are therefore now recommended for this patient
66 group (18).

67 In 2018, resistance to TZP occurred in 9.1% of invasive *E. coli* isolates in the UK (19). This can be caused by the
68 production of carbapenemase enzymes (20), multiple β-lactamases (21) or ESBLs in combination with increased
69 efflux or porin loss (22), which also provide resistance to 3GCs. Recently, a phenotype of resistance to TZP with

70 susceptibility to 3GCs (TZP-R/3GC-S) emerged in *E. coli* and *Klebsiella pneumoniae*, indicating the possibility of
71 alternative resistance mechanisms. The major cause of this phenotype is the hyperproduction of class A or D β-
72 lactamases such as TEM-1 (23, 24). Increased production of β-lactamase overcomes the inhibitive effect of
73 tazobactam, ostensibly through saturation of the inhibitor, allowing the excess enzyme to hydrolyse and
74 degrade piperacillin (25). β-lactamase hyperproduction can occur via increased gene expression modulated by
75 a stronger promoter (26), or an increase in gene copy number mediated by insertion sequences (27, 28) or
76 plasmids (24) . Other mechanisms have also been identified, including expression of OXA-1 (25), inhibitor
77 resistant enzymes such as $bla_{\text{TEM-33 (11)}}$, and a single nucleotide polymorphism (SNP) at position S133G in $bla_{\text{CTX-M-}}$
78 $_{15}$ found *in vitro* via random mutagenesis/error prone PCR but not yet found in clinical isolates (29).
79 Routine blood culture surveillance identified the occurrence of this phenotype in *E. coli* at the Royal Liverpool
80 University Hospital (RLUH), Liverpool, UK, between 2014 and 2017. We sought to identify the diversity of *E. coli*
81 strains and distribution of known mechanisms of TZP. We compared our collection to the findings of a UK-wide
82 collection of invasive *E. coli* isolates (*n* = 1509) with publicly available phenotypic and genotypic data. This data
83 set included the TZP-R/3GC-S phenotype as well as a piperacillin/tazobactam and third generation
84 cephalosporins susceptible (TZP-S/3GC-S) phenotype

85 **Methods**

86 *Study setting*

87 The RLUH is a city centre located hospital in Liverpool, UK, providing secondary and tertiary care, with a
88 catchment area of >2 million people in Merseyside, Cheshire, North Wales, and the Isle of Man. In 2019 the
89 hospital recorded over 587,000 outpatient appointments and 95,000 daily inpatients.

90 *Ethics statement*

91 The study utilised bacterial isolates collected by the RLUH for standard diagnostic purposes. All isolates were
92 anonymised and de-linked from patient data. As no human samples or patient data were utilised in the study,

93 ethical approval was not required. This was confirmed using the online NHS REC review tool http://www.hra-
94 decisiontools.org.uk/ethics/.

95 *Surveillance data & Isolate collection*

96 Blood stream bacterial pathogens were isolated using the BacTAlert 3D blood culture system (bioMérieux,
97 France) and identified to a species level using MALDI-TOF (Bruker, US). Antimicrobial susceptibility testing (AST)
98 was carried out using disk diffusion-based testing according to the British Society of Antimicrobial
99 Chemotherapy guidelines (30) between 2014 and August 7$^{th}$ 2017, after which these were replaced by the
100 European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines (31). In 2014 ceftazidime was
101 used as the indicator 3GC, which was changed to cefpodoxime between 2015 and 2017. Isolate details and AST
102 results were recorded in the Laboratory Information System (Telepath, CSC, US). All isolates were retained in
103 glycerol stocks at -80°C in the RLUH Biobank. Data for the study was extracted into a database, including
104 susceptibility data for ampicillin, cefpodoxime/ceftazidime, TZP, meropenem, ertapenem, cefoxitin,
105 ciprofloxacin, gentamycin, amikacin, amoxicillin/clavulanic acid, tigecycline, and chloramphenicol. The data was
106 used to estimate the proportion of *E. coli* isolates per year with TZP resistance, with and without associated
107 3GC resistance. In cases where multiple isolates were obtained from a single infectious episode, only the first
108 isolate was included for further investigation and sequencing, to avoid duplication. Isolates that were TZP-
109 R/3GC-S were retrieved from the Biobank and resurrected from glycerol stocks using Luria-Bertani agar (Oxoid,
110 UK) and incubated at 37$^{o}$C for 18 hours.

111 *Antimicrobial Susceptibility*

112 Minimum inhibitory concentrations (MIC) for the isolates were obtained using the E-TEST method (Biomerieux,
113 France) (32) according to EUCAST guidelines. (33) MICs were determined for TZP and the 3GC ceftriaxone
114 (CTX).

115 *DNA extraction and sequencing*

116 Genomic DNA was extracted using the PureGene Yeast/Bacteria Kit (Qiagen, Germany), following the
117 manufacturer's instructions for Gram-negative bacteria. Genome sequencing of 65 isolates was performed by
118 MicrobesNG (http://www.microbesng.uk), using 2 x 250 bp short-read sequencing on the Illumina MiSeq
119 (Illumina, US) (Table S1).

120 *Genome analysis, sequence typing and AMR gene prediction*

121 All genomes were *de novo* assembled and annotated using SPAdes version 3.7 (34), and Prokka 1.11 (35),
122 respectively, by MicrobesNG, in addition to providing the trimmed and quality filtered sequencing reads. The
123 presence and copy number of AMR genes was determined using ARIBA (36), with the SRST2 database (37). *In*
124 *silico* multi locus sequence typing (MLST), and plasmid replicon typing were carried out using ARIBA and the
125 MLSTFinder (38) and PlasmidFinder (39) databases, respectively. β-lactamase promoters were identified by
126 constructing databases with promoter sequences for $bla_{\text{TEM-1}}$ (26) and screening using ARIBA . Copy numbers
127 were estimated by dividing the sequencing coverage of β-lactamase genes by the coverage of the
128 chromosomal single copy gene *ampH*.

129 *Phylogenetic analysis of study isolates*

130 A pan-genome analysis of all sequences was generated using Roary (40), and the core gene alignment was used
131 as input for snp-sites (41) to extract ACGT-only SNPs (-c option). A maximum likelihood tree was produced
132 using iqtree (42), with the general time reversible (GTR) model and gamma correction using ASC ascertainment
133 bias correction (ASC) for SNPs-only alignments (-m GTR+G+ASC) and 1000 bootstrap replicates (-bb 1000).
134 Phylogenetic trees were annotated using the Interactive Tree of Life (43) (https://itol.embl.de/). Core genome
135 trees for sequence types ST131 and ST73 were generated by mapping the reads against the reference
136 chromosomes of *E. coli* strains EC958 (HG941718.1) and CFT073 (AE014075.1), respectively, using snippy
137 (https://github.com/tseemann/snippy). Recombination blocks were removed with Gubbins (44), and extraction
138 of SNPs-only of the recombination-free alignment, and tree calculation, were performed as described above,
139 using SNP-sites and IQ-TREE.

140 To investigate the relation of the study isolates to the whole UK hospital *E. coli* population, the sequences from
141 a large UK-wide comparative analysis were included (PRJEB4681, (45)). These sequences included 1094 isolates
142 submitted to the UK wide Bacteraemia Resistance Surveillance Programme (www.bsacsurv.org) between
143 2001–2011 by 11 hospitals across England, and 415 isolates provided by the Cambridge University Hospitals
144 NHS Foundation Trust, Cambridge.

145 A core gene alignment and phylogenetic tree were constructed. Isolates from the UK-wide collection with the
146 same phenotype of TZP resistance/3GC susceptibility (defined as susceptibility to both ceftazidime and
147 cefotaxime, or either compound if only one was tested) were identified from the phenotypic AMR data (45),
148 and highlighted alongside study isolates.

149 *Data availability*

150 Raw read data and assemblies were submitted under BioProject ID PRJNA644114. Detailed per-strain
151 information on accession numbers, resistance profiles, resistance gene predictions and sequence types (STs)
152 are given in Table S1.

153 **Results**

154 ***Isolate collection and antimicrobial susceptibility testing***

155 The RLUH recorded 1472 BSI *E. coli* isolates between 2014 and 2017 and antimicrobial susceptibility testing
156 showed 172 isolates (11.8%) were resistant to TZP (Fig.S1). The proportion of *E. coli* resistant to TZP declined
157 between 2014 (21%) and 2017 (9%, Fig. 1C). Of the 1258 TZP-susceptible isolates, the majority (1129, 89.7%)
158 were susceptible to 3GC, while 129 (10.3%) were 3GC non-susceptible. In contrast, 86/172 (50%) TZP-resistant
159 isolates were non-susceptible and 86/172 (50%) were susceptible to 3GC (Fig.1A).

160 Resistance to carbapenems was only seen in the TZP-resistant/3GC-resistant isolates, with 3.9% resistant to
161 meropenem and 5.3% to ertapenem. A higher proportion of the TZP-R/3GC-S isolates were resistant to
162 amoxicillin/clavulanic acid in comparison with TZP-resistant/3GC-resistant isolates (96.4% vs 81.1%) (Fig.1B).

163 Overall, aside from the penicillin class antibiotics, the TZP-R/3GC-S phenotype had high incidence of
164 susceptibility towards the antimicrobials tested.

165 Of the 86 isolates with the TZP-R/3GC-S phenotype, 14 had been derived from repeated sampling of long-term
166 patients and were excluded, resulting in 72 isolates derived from unique patients. These isolates were reduced
167 to 66 after excluding TZP MICs under the EUCAST breakpoint for susceptibility. A further isolate was considered
168 a contaminant (*Staphylococcus aureus*) based on colony morphology, which was confirmed by 16S PCR. After
169 whole genome sequencing, two of the 65 isolates were removed as they contained more than one *E. coli*
170 genome, either due to mixed infections or contamination (assembly sizes were 9602556bp and 9552068bp,
171 respectively), leaving 63 isolates for further analysis.

172 The MICs of TZP as assessed by the E-TEST ranged from 12 to 256 mg/L. Fifty-eight isolates had MICs over the
173 EUCAST breakpoint for resistance (16 mg/L), and five had intermediately resistance (MIC 12mg/L). The CTX
174 MICs ranged between 0.016 and 0.25 mg/L, all below the breakpoint for resistance (2mg/L), confirming the
175 TZP-R/3GC-S phenotype.

176 ***Resistance and plasmid profile of TZP-resistant/3GC-susceptible population***

177 The AMR genotypes (Fig.S2) correlated well with the phenotypic data obtained by disk testing, with most
178 isolates susceptible to ciprofloxacin and gentamicin. The 58 TZP-R/3GC-S isolates harboured a variety of β-
179 lactamase genes, including TEM-type (n=44; $bla_{\text{TEM-1}}$ [41], $bla_{\text{TEM-33}}$ [2], $bla_{\text{TEM-148}}$ [1]), $bla_{\text{SHV-1}}$ (n=9), $bla_{\text{CTX-M-15}}$
180 (n=4) and $bla_{\text{OXA-1}}$ (n=3)).The presence of β-lactamase genes correlated with resistance to ampicillin and TZP,
181 whilst resistance to ciprofloxacin in 17/58 isolates (29%) was accounted for by *gyrA* mutations D87N (10/17,
182 59%) and S83L (12/17, 71%),and *parC* S80I mutation (10/17, 59%). Aminoglycoside resistance was explained by
183 the *O*-adenylyltransferases *aadA* (6/6, 100%), in combination with the genes *aac(3)-IIa* or *aadB* (3/6, 50%).
184 Additionally, all isolates carried the chromosomal $bla_{\text{AmpC1}}$ which is constitutively expressed at a low level (46),
185 and 51/58 of the isolates carried $bla_{\text{AmpC2}}$ .A single isolate (169961) had a coding mutation in a penicillin binding
186 protein, with an A37T mutation in *mrdA* encoding penicillin binding protein 2, in combination with the inhibitor
187 resistant $bla_{\text{TEM-33}}$ and strong *Pa/Pb* promoter.

188 Replicons usually associated with large resistance plasmids, such as IncFIA and IncFIB, IncFIA and IncFIIA, were
189 detected in 19% of the study isolates (Fig.2), reflecting the low proportion of isolates with multiple resistance
190 genes and the unusual resistance profile characteristic of the TZP-R/3GC-S phenotype.

191 ***Population structure of the TZP-resistant/3GC-susceptible population within the nationwide context***

192 Phylogenetic analysis revealed the TZP-R/3GC-S phenotype occurred in a diverse number of sequence types
193 (Fig.2). The 58 TZP-R/3GC-S isolates represented 16 STs. The most representative were ST131 (36.2%), ST73
194 (19%) and ST12 (6.9%). The TZP-R/3GC-S phenotype in the UK-wide collection was similarly diverse to the RLUH
195 collection with ST131 (22.1%) the most represented, followed by ST73 (16.2%) and ST95 (14.7%, Fig.3). This
196 diversity was also reflected in the TZP-S/3GC-S phenotype; ST73 (16.8%), ST131 (14.3%) and ST95 (10.6%).
197 When placing the RLUH isolates into the phylogenetic context of the UK-wide bloodstream isolates collected
198 from 2001 to 2011, it was apparent that they reflected the overall *E. coli* population structure (Fig.4). This
199 indicates that the TZP-R/3GC-S phenotype is not driven by a clonal outbreak within this single hospital setting,
200 but rather by multiple acquisitions of resistance mechanisms in the circulating population of hospital strains.
201 The AMR gene profile of the RLUH isolates varied between STs (Fig.S3), with ST131 carrying more resistance
202 genes than the other major STs, as previously reported (47). To get a higher-resolution insight into the within-
203 ST diversity of the isolates, we calculated core genome trees of the main STs by mapping the reads against
204 selected reference genomes and extracting the conserved, non-recombinant SNPs. The acquisition of the
205 phenotype was not a single event even in these closely related organisms, as it occurred on several occasions
206 for both main sequence types, with no (ST73; Fig. S4) or very few (ST131; Fig. S5) isolates closely related, which
207 may indicate within-hospital transmission.

208

209 ***Varied putative genetic determinants of the TZP-resistant/3GC-susceptible phenotype***

210 We sought to identify previously published putative resistance mechanisms associated with the TZP-R/3GC-S
211 phenotype in the 58 isolates from RLUH (Fig. 5A). No carbapenemase genes were predicted to be present,

212 although four ST131 isolates harboured the ESBL *bla*$_{CTX-M-15}$ gene, normally associated with 3GC resistance.
213 However, three isolates carried a SNP resulting in the non-synonymous amino acid change from serine to
214 glycine at position 133. This amino acid change is reported to result in a non-ESBL phenotype with increased
215 TZP resistance. However the S133G mutation was only identified through random mutagenesis/error prone
216 PCR *in vitro* (29). In the remaining isolate with *bla*$_{CTX-M-15}$ the promoter sequence was deleted and therefore
217 presumably not expressed (Fig, S6). However, the isolate also carried *bla*$_{OXA-1}$. In all three isolates carrying
218 *bla*$_{OXA-1}$, it was either the sole β-lactamase or it was carried with a second β-lactamase. Of the isolates with
219 *bla*$_{TEM-1}$, 25 had the weak *P3* promoter, four had the strong promoter *P4* and 12 contained the strong,
220 overlapping promoter *Pa/Pb*. The *P4* and *Pa/Pb* promoter have previously been linked to hyperproduction of
221 TEM-1 (26). The TZP-R/3GC-S phenotype has previously been associated with increases in the copy number of
222 *bla*$_{TEM-1}$ via gene amplification, resulting in hyperproduction of the TEM-1 enzyme (23, 27). The copy numbers
223 of *bla*$_{TEM-1}$, as estimated by sequencing coverage, for those isolates within the RLUH collection with a weak *P3*
224 promoter varied between 3 and 186 copies, and a mean of 44 copies (Fig. 5B).
225 We identified inhibitor resistant β-lactamases (3; 4%), *bla*$_{OXA-1}$ (10, 14.7%) and *bla*$_{TEM-1}$ promoter region
226 mutations (18; 26%) in the 68 TZP-R/3GC-S isolates from the UK wide collection (Fig. 5A). However, we also
227 identified these mechanisms, although at a lower incidence (inhibitor resistant β-lactamase; 2 [0.2%], *bla*$_{OXA-1}$
228 24 [1.8%] promoter region mutations; 89 [7%]), in the TZP-S/3GC-S phenotype in the same collection. In total a
229 putative mechanism was found in 27 of the 58 isolates.
230 The copy number of *bla*$_{TEM-1}$ was also elevated in both the TZP-R/3GC-S (min-max of 0.5 and 129 copies, mean
231 16 copies) and TZP-S/3GC-S (min-max of 0.02 and 68 copies, mean 3 copies) from the UK wide collection (Fig.
232 5B). Despite this, there was a significant difference in copy number between the TZP-S/3GC-S vs UK wide TZP-
233 R/3GC-S phenotypes (P value < 0.001; Dunn's Multiple Comparison Test) and the TZP-S/3GC-S vs TZP-R/3GC-S
234 phenotypes from RLUH (P value < 0.001; Dunn's Multiple Comparison Test). This indicates that although an
235 increase in copy number of *bla*$_{TEM-1}$ may not be predictive of TZP-R/3GC-S, it is associated with the phenotype.

236 **Discussion**

237 This phylogenetic analysis of the TZP-R/3GC-S phenotype in *E. coli* from RLUH demonstrates that this
238 phenotype derives from repeated, multiple acquisition events. Our comparison of the RLUH isolates with a
239 large UK-wide collection (45) shows that this is not unique to our study site, but broadly reflective of the
240 phenotype from multiple sites across the UK. As the TZP-R/3GC-S phenotype also reflects the overall UK
241 population structure of *E. coli* bacteraemia isolates, this is suggestive of the impact of repeated or sustained
242 antimicrobial pressure, rather than fixation in a certain lineage and subsequent spread. The phenotype was
243 encountered in the typically drug resistant ST131 (48), and the often highly virulent but drug susceptible ST73
244 (49), reflecting the overall dominance of these STs, and was not associated with an overall increase in carriage
245 of genes conferring resistance to other classes of antibiotics. We also were unable to identify the presence of a
246 common plasmid replicon, further highlighting the diversity of the phenotype.

247 Strategies to increase the effectiveness of TZP include increasing dosage, which in one study increased the
248 coverage of TZP from 83.2% to 93% of bacterial blood stream pathogens (50). Increasing the concentration of
249 tazobactam alongside a fixed dose of piperacillin has also rescued TZP effectiveness against TEM-1
250 hyperproducers in a neutropenic mouse model (25), and could be a viable strategy to protect its future
251 effectiveness. It is worth noting observational clinical data (51), and *in vivo* experimental data (52), suggesting
252 TZP may be effective against some organisms with *in vitro* phenotypic resistance to TZP.

253 The rapid identification of the TZP-R/3GC-S phenotype would enable de-escalation from TZP to a 3GC (53),
254 both reducing the likelihood of treatment failure, and preventing overuse of carbapenems, which is key for
255 antimicrobial stewardship (54). The isolates were also mostly susceptible to ciprofloxacin, gentamicin and
256 amikacin, providing further de-escalation opportunities. Recent work on methicillin resistant *Staphylococcus*
257 *aureus* has described frequent collateral sensitivity to narrow spectrum penicillin/inhibitor combinations,
258 highlighting that targeted de-escalation rather than escalation can be possible when treating organisms highly
259 resistant to first line drugs (55). Molecular diagnostics and whole genome sequencing can be used to rapidly
260 detect AMR genes to predict AMR phenotype (56, 57). However, it is essential to match the phenotypic

261 resistance to the genotypic mechanisms. The majority of TZP-R/3GC-S isolates in this study hyperproduced the
262 class A β-lactamase enzymes *bla*$_{TEM-1}$, which can hydrolyse piperacillin but not 3GCs, and is inhibited by
263 tazobactam. Hyperproduction can occur via gene amplification, in which tandem repeats of AMR genes are
264 generated, for example via the IS*26* mediated amplification of pseudo-compound transposons (27, 58), or the
265 transfer of β-lactamase genes to high copy AMR plasmids (24). A number of the isolates were lacking a
266 detectable increase in gene copy number, but had a potential route to hyperproduction via a strong promoter
267 of *bla*$_{TEM-1}$ (26).
268 We also detected *bla*$_{TEM-33}$, encoding an inhibitor resistant variant of TEM-1B (59), and *bla*$_{OXA-1}$, either as the
269 only β-lactamase or in combination with *bla*$_{TEM-1}$. OXA-1 is poorly inhibited by tazobactam (60) but has been
270 associated with the TZP-R/3GC-S phenotype (61), while a recent UK study identified *bla*$_{OXA-1}$ as a major
271 contributor to TZP resistance amongst ESBL *E. coli* (61). However, the carriage of *bla*$_{OXA-1}$ does not always confer
272 resistance to TZP, which appears to depend on the genetic background of the strain. The risk ratio of *bla*$_{OXA-1}$
273 being associated with TZP resistance in ESBL *E. coli* is higher in ST131 strains (12.1) compared with ESBL *E. coli*
274 as a whole (6.49) (61). One isolate carrying the OXA-1 β-lactamase gene, as well as *bla*$_{CTX-M-15}$ lacking a
275 promoter, belonged to ST131.
276 Three out of four detected *bla*$_{CTX-M-15}$ encoded the S133G mutation, which increases TZP MIC ten-fold, whilst
277 reducing the 3GC MIC by the same margin in a strain harbouring a random mutagenesis/error prone PCR
278 derived *bla*$_{CTX-M-15}$(29). To our knowledge this is the first report of this *bla*$_{CTX-M-15}$ variant in clinical isolates. The
279 S133G mutation in *bla*$_{CTX-M-15}$ was associated with 5% of TZP-R/3GC-S in our setting and only in ST131. The
280 mutation of *bla*$_{CTX-M}$ genes to better hydrolyse mecillinam has been reported during urinary tract infections
281 treatment (62), but not for TZP or other β-lactam/inhibitor combinations. The circulation of *bla*$_{CTX-M}$ variants
282 that do not confer the ESBL phenotype but provide resistance to TZP, has implications for molecular testing for
283 ESBL organisms (63), as it would misclassify the isolates as 3GC-resistant and lead to unnecessary use of
284 carbapenems. There is thus a need for *in vitro* development of resistant mutations to uncover potential routes
285 to resistance and improve AMR prediction. We found that 9 of 58 isolates, all without a putative resistant

286 mechanism, harboured blaSHV-48. Hyperproduction of this enzyme has been shown to lead to the TZP-R/3GC-
287 S phenotype in *Klebsiella pneumoniae* (64).

288 All the putative mechanisms of TZP-R/3GC-S found in the isolates from RLUH have been previously published
289 and widely associated with this phenotype. However, we found evidence of $bla_{TEM-1}$ promoter region
290 mutations, inhibitor resistance enzymes and increased $bla_{TEM-1}$ copy number in the TZP-S/3GC-S phenotype. The
291 only putative mechanism which was not found in the TZP-S/3GC-S phenotype was the S133G mutation in $bla_{CTX-}$
292 $_{M-15}$. This mutation was not found in any of the TZP-R/3GC-S phenotype isolates from the UK wide collection,
293 which may indicate low incidence or a localised emergence in our hospital. The diverse putative mechanisms of
294 TZP-R/3GC-S and phenotype-genotype discordance, as seen in TZP-S/3GC-S, would compromise current
295 molecular or genomic detection of this phenotype.

296 The main limitation of this study was that only TZP-R/3GC-S isolates from the RLUH were sequenced, and the
297 relatively small population size. We utilised a large and UK-wide collection of isolates for comparison, which
298 were similarly diverse and reflected the overall population structure (45).

299 This work highlights the phylogenetic diversity of the TZP-R/3GC-S phenotype in *E. coli* and the variety of the
300 putative resistance mechanisms involved, including β-lactamase hyperproduction via gene amplification and
301 promoter mutations, inhibitor resistant TEM-1 and CTX-M-15 variants. However, the presence of these
302 mechanisms at a lower incidence with the TZP-S isolates highlights that a greater understanding of the
303 evolution of TZP resistance and the resistance mechanisms of the TZP-R/3GC-S phenotype would be
304 fundamental to improve the prediction of TZP-R/3GC-S *E. coli*. Until such time, phenotypic monitoring of this
305 phenotype is essential to prevent treatment failure.

306 **Funding**

307 This work was supported by the LSTM Director's Catalyst Fund, separately awarded to TE and ATMH. EH
308 acknowledges support from a Wellcome SEED Award (217303/Z/19/Z). APR would like to acknowledge funding
309 from the AMR Cross-Council Initiative through a grant from the Medical Research Council, a Council of UK

310 Research and Innovation (Grant Number; MR/S004793/1), and the National Institute for Health Research
311 (Grant number; NIHR200632).

312 **Author contributions**

313 TE, EH, JM, CMP and ATMH conceptualised the study. JvA, AH PR, CC, CMP, JM, and AH collated isolate
314 metadata, and clinical antimicrobial susceptibility testing data. TE, EH, ERA, APR, LEC and ATMH contributed to
315 the experimental design and data analysis. Bioinformatic analysis was carried out by TE and EH. TE, JvA, CTW,
316 AJF, IB and ATMH carried out microbiological experiments. TE, EH and ATMH wrote the first draft of the
317 manuscript. All authors reviewed and edited the final manuscript.

318 **Acknowledgments**

319 We acknowledge the technical staff in the diagnostic microbiology laboratories in the RLUH and expert
320 informatics support from the Pathogen Informatics team at the Wellcome Sanger Institute.

321 **Competing Interests**

322 The authors declare no competing interests.

323 **Additional information**

324 Correspondence and requests for materials should be addressed to TE or ATMH.

325 **References**

326 1. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. *Clin. Microbiol.*
327 *Infect.* 2013;19(6):492-500.
328 2. Bonten M, Johnson JR, van den Biggelaar AHJ, Georgalis L, Geurtsen J, de Palacios PI, et al.
329 Epidemiology of Escherichia coli Bacteremia: A Systematic Literature Review. *Clin. Infect. Dis.* 2020.
330 3. Public Health England. Annual epidemiological commentary: mandatory MRSA, MSSA and E coli
331 bacteraemia and C difficile infection data. 2014.
332 4. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research,
333 discovery, and developmentof new antibiotics. 2017.
334 5. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and
335 development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet*
336 *Infect. Dis.* 2018;18(3):318-27.
337 6. World Health Organization. Landscape of diagnostics against antibacterial resistance, gaps and
338 priorities. 2019.
339 7. Temkin E, Fallach N, Almagor J, Gladstone BP, Tacconelli E, Carmeli Y. Estimating the number of
340 infections caused by antibiotic-resistant Escherichia coli and Klebsiella pneumoniae in 2014: a modelling study.
341 *Lancet Glob. health.* 2018;6(9):e969-e79.

342 8. European Centres of Disease Control. Surveillance of antimicrobial resistance in Europe 2018
343 2018.
344 9. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. β-Lactamases
345 and β-Lactamase Inhibitors in the 21st Century. *J Mol Biol.* 2019;431(18):3472-500.
346 10. Gin A, Dilay L, Karlowsky JA, Walkty A, Rubinstein E, Zhanel GG. Piperacillin-tazobactam: a beta-
347 lactam/beta-lactamase inhibitor combination. Expert review of anti-infective therapy. 2007;5(3):365-83.
348 11. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. *Clin. Microbiol. Rev*.
349 2010;23(1):160-201.
350 12. Johnson DM, Biedenbach DJ, Jones RN. Potency and antimicrobial spectrum update for
351 piperacillin/tazobactam (2000): emphasis on its activity against resistant organism populations and generally
352 untested species causing community-acquired respiratory tract infections. *Diagn. microbiol. Infect. Dis*.
353 2002;43(1):49-60.
354 13. Kim M-K, Xuan D, Quintiliani R, Nightingale CH, Nicolau DP. Pharmacokinetic and pharmacodynamic
355 profile of high dose extended interval piperacillin–tazobactam. *J. Antimicrob. Chemother*. 2001;48(2):259-67.
356 14. Barton GJ, Morecroft CW, Henney NC. A survey of antibiotic administration practices involving patients
357 with sepsis in UK critical care units. *Int J Clin Pharm*. 2020;42(1):65-71.
358 15. Wilson APR. Sparing carbapenem usage. Journal of Antimicrobial Chemotherapy. 2017;72(9):2410-7.
359 16. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment Options for Carbapenem-Resistant
360 Enterobacteriaceae Infections. *Open Forum Infect. Dis.* 2015;2(2):ofv050.
361 17. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of Piperacillin-Tazobactam vs
362 Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and
363 Ceftriaxone Resistance: A Randomized Clinical Trial. *Jama*. 2018;320(10):984-94.
364 18. Gutiérrez-Gutiérrez B, Rodríguez-Baño J. Current options for the treatment of infections due to
365 extended-spectrum beta-lactamase-producing Enterobacteriaceae in different groups of patients. Clin.
366 Microbiol. Infect. 2019;25(8):932-42.
367 19. Public Health England. Laboratory surveillance of Escherichia coli bacteraemia in England, Wales and
368 Northern Ireland. Health Protection Report. 2018;13(37).
369 20. van Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-
370 Lactam/β-Lactamase Inhibitor Combinations. *Clin. infect. Dis.* 2016;63(2):234-41.
371 21. Lee J, Oh CE, Choi EH, Lee HJ. The impact of the increased use of piperacillin/tazobactam on the
372 selection of antibiotic resistance among invasive Escherichia coli and Klebsiella pneumoniae isolates. *Int J Infect*
373 *Dis.* 2013;17(8):e638-43.
374 22. Suzuki Y, Sato T, Fukushima Y, Nakajima C, Suzuki Y, Takahashi S, et al. Contribution of beta-lactamase
375 and efflux pump overproduction to tazobactam-piperacillin resistance in clinical isolates of Escherichia coli. *Int.*
376 *J. Antimicrob. Agents.* 2020:105919.
377 23. Schechter LM, Creely DP, Garner CD, Shortridge D, Nguyen H, Chen L, et al. Extensive Gene
378 Amplification as a Mechanism for Piperacillin-Tazobactam Resistance in Escherichia coli. mBio. 2018;9(2).
379 24. Rodríguez-Villodres Á, Gil-Marqués ML, Álvarez-Marín R, Bonnin RA, Pachón-Ibáñez ME, Aguilar-
380 Guisado M, et al. Extended-spectrum resistance to β-lactams/β-lactamase inhibitors (ESRI) evolved from low-
381 level resistant Escherichia coli. *J. Antimicrob. Chemother.* 2019;75(1):77-85.
382 25. Abdelraouf K, Chavda KD, Satlin MJ, Jenkins SG, Kreiswirth BN, Nicolau DP. Piperacillin-Tazobactam-
383 Resistant/Third-Generation Cephalosporin-Susceptible Escherichia coli and Klebsiella pneumoniae Isolates:
384 Resistance Mechanisms and In vitro-In vivo Discordance. *Int. J. Antimicrob. Agents*. 2020;55(3):105885.
385 26. Zhou K, Tao Y, Han L, Ni Y, Sun J. Piperacillin-Tazobactam (TZP) Resistance in Escherichia coli Due to
386 Hyperproduction of TEM-1 β-Lactamase Mediated by the Promoter Pa/Pb. *Front Microbiol*. 2019;10:833-.
387 27. Hubbard ATM, Mason J, Roberts P, Parry CM, Corless C, van Aartsen J, et al. Piperacillin/tazobactam
388 resistance in a clinical isolate of Escherichia coli due to IS*26*-mediated amplification of $bla_{\text{TEM-1B}}$. *Nat Comms*.
389 2020;4915

390 28. Hansen KH, Andreasen MR, Pedersen MS, Westh H, Jelsbak L, Schønning K. Resistance to
391 piperacillin/tazobactam in Escherichia coli resulting from extensive IS 26-associated gene amplification of bla
392 TEM-1. *J. Antimicrob. Chemother.* 2019;74(11):3179-83.
393 29. Rosenkilde CEH, Munck C, Porse A, Linkevicius M, Andersson DI, Sommer MOA. Collateral sensitivity
394 constrains resistance evolution of the CTX-M-15 β-lactamase. Nature Communications. 2019;10(1):618.
395 30. Andrews JM, Howe RA. BSAC standardized disc susceptibility testing method (version 10). *J.*
396 *Antimicrob. Chemother.* 2011;66(12):2726-57.
397 31. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of
398 MICs and zone diameters. 2013;5.
399 32. García-Fernández S, Bala Y, Armstrong T, García-Castillo M, Burnham C-AD, Wallace MA, et al.
400 Multicenter Evaluation of the New Etest Gradient Diffusion Method for Piperacillin-Tazobactam Susceptibility
401 Testing of Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii Complex. *J. Clin.*
402 *Microbiol.* 2020;58(2):e01042-19.
403 33. European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoints - bacteria (v10.0).
404 https://wwweucastorg/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_100_Breakpoint_Tablesp
405 df. 2020.
406 34. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome
407 assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 2012;19(5):455-77.
408 35. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics.* 2014;30(14):2068-9.
409 36. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial
410 resistance genotyping directly from sequencing reads. *Microb. Genom.* 2017;3(10):e000131-e.
411 37. Inouye M, Dashnow H, Raven L-A, Schultz MB, Pope BJ, Tomita T, et al. SRST2: Rapid genomic
412 surveillance for public health and hospital microbiology labs. *Genom. Med.* 2014;6(11):90.
413 38. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing
414 of total-genome-sequenced bacteria. *J. Clin . Microbiol.* 2012;50(4):1355-61.
415 39. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and
416 typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents*
417 *Chemother.* 2014;58(7):3895-903.
418 40. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large-scale
419 prokaryote pan genome analysis. *Bioinformatics.* 2015;31(22):3691-3.
420 41. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction
421 of SNPs from multi-FASTA alignments. *Microb. Genom.* 2016;2(4):e000056.
422 42. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for
423 estimating maximum-likelihood phylogenies. Molecular biology and evolution. 2015;32(1):268-74.
424 43. Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of
425 phylogenetic and other trees. *Nucleic Acids Res.* 2016;44(W1):W242-5.
426 44. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of
427 large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res.*
428 2015;43(3):e15.
429 45. Kallonen T, Brodrick HJ, Harris SR, Corander J, Brown NM, Martin V, et al. Systematic longitudinal
430 survey of invasive Escherichia coli in England demonstrates a stable population structure only transiently
431 disturbed by the emergence of ST131. *Genome Res.* 2017;27(8):1437-49.
432 46. Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, Böttger EC, et al. Detection of AmpC beta-
433 lactamase in Escherichia coli: comparison of three phenotypic confirmation assays and genetic analysis. *J. Clin.*
434 *Microbiol.* 2011;49(8):2924-32.
435 47. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, et al. Diversification of
436 Colonization Factors in a Multidrug-Resistant Escherichia coli Lineage Evolving under Negative Frequency-
437 Dependent Selection. *mBio.* 2019;10(2):e00644-19.

438 48. Whitmer GR, Moorthy G, Arshad M. The pandemic Escherichia coli sequence type 131 strain is acquired
439 even in the absence of antibiotic exposure. *PLOS Pathogens.* 2019;15(12):e1008162.
440 49. Cunha MPV, Saidenberg AB, Moreno AM, Ferreira AJP, Vieira MAM, Gomes TAT, et al. Pandemic extra-
441 intestinal pathogenic Escherichia coli (ExPEC) clonal group O6-B2-ST73 as a cause of avian colibacillosis in Brazil.
442 *PLOS ONE*. 2017;12(6):e0178970.
443 50. Rothe K, Wantia N, Spinner CD, Schneider J, Lahmer T, Waschulzik B, et al. Antimicrobial resistance of
444 bacteraemia in the emergency department of a German university hospital (2013–2018): potential
445 carbapenem-sparing empiric treatment options in light of the new EUCAST recommendations. *BMC Infect. Dis.*
446 2019;19(1):1091.
447 51. Baker TM, Rogers W, Chavda KD, Westblade LF, Jenkins SG, Nicolau DP, et al. Epidemiology of
448 Bloodstream Infections Caused by Escherichia coli and Klebsiella pneumoniae That Are Piperacillin-
449 Tazobactam-Nonsusceptible but Ceftriaxone-Susceptible. *Open Forum Infect Dis.* 2018;5(12):ofy300.
450 52. Stainton SM, Monogue ML, Nicolau DP. In Vitro-In Vivo Discordance with Humanized Piperacillin-
451 Tazobactam Exposures against Piperacillin-Tazobactam-Resistant/Pan-β-Lactam-Susceptible Klebsiella
452 pneumoniae Strains. *Antimicrob Agents Chemother.* 2017;61(7).
453 53. Carlisle L, Justo JA, Al-Hasan MN. Bloodstream Infection due to Piperacillin/Tazobactam Non-
454 Susceptible, Cephalosporin-Susceptible Escherichia coli: A Missed Opportunity for De-Escalation of Therapy.
455 *Antibiotics*. 2018;7(4).
456 54. Robson SE, Cockburn A, Sneddon J, Mohana A, Bennie M, Mullen AB, et al. Optimizing carbapenem use
457 through a national quality improvement programme. *J. Antimicrob. Chemother.* 2018;73(8):2223-30.
458 55. Harrison EM, Ba X, Coll F, Blane B, Restif O, Carvell H, et al. Genomic identification of cryptic
459 susceptibility to penicillins and β-lactamase inhibitors in methicillin-resistant Staphylococcus aureus. *Nat.*
460 *Microbiol.* 2019;4(10):1680-91.
461 56. Williams CT, Edwards T, Adams ER, Feasey NA, Musicha P. ChloS-HRM, a novel assay to identify
462 chloramphenicol-susceptible Escherichia coli and Klebsiella pneumoniae in Malawi. *J. Antimicrob. Chemother.*
463 2019.
464 57. Charalampous T, Kay GL, Richardson H, Aydin A, Baldan R, Jeanes C, et al. Nanopore metagenomics
465 enables rapid clinical diagnosis of bacterial lower respiratory infection. *Nat. Biotechnol.* 2019;37(7):783-92.
466 58. Harmer CJ, Pong CH, Hall RM. Structures bounded by directly-oriented members of the IS26 family are
467 pseudo-compound transposons. *Plasmid.* 2020;111:102530.
468 59. Sirot D, Chanal C, Bonnet R, De Champs C, Bret L. Inhibitor-resistant TEM-33 beta-lactamase in a
469 Shigella sonnei isolate. *Antimicrob. Agents Chemother.* 2001;45(7):2179-80.
470 60. Che T, Bethel CR, Pusztai-Carey M, Bonomo RA, Carey PR. The different inhibition mechanisms of OXA-
471 1 and OXA-24 beta-lactamases are determined by the stability of active site carboxylated lysine. *J. Biol. Chem.*
472 2014;289(9):6152-64.
473 61. Livermore DM, Day M, Cleary P, Hopkins KL, Toleman MA, Wareham DW, et al. OXA-1 β-lactamase and
474 non-susceptibility to penicillin/β-lactamase inhibitor combinations among ESBL-producing Escherichia coli. *J.*
475 *Antimicrob. Chemother.* 2018;74(2):326-33.
476 62. Nielsen KL, Hansen KH, Nielsen JB, Knudsen JD, Schønning K, Frimodt-Møller N, et al. Mutational
477 change of CTX-M-15 to CTX-M-127 resulting in mecillinam resistant Escherichia coli during pivmecillinam
478 treatment of a patient. *MicrobiologyOpen*. 2019;8(12):e941.
479 63. Edwards T, Williams C, Teethaisong Y, Sealey J, Sasaki S, Hobbs G, et al. A highly multiplexed melt-curve
480 assay for detecting the most prevalent carbapenemase, ESBL and AmpC genes. *Diag. Microbiol. Infect. Dis.*
481 2020:115076.
482 64. Han MS, Park KS, Jeon JH, Lee JK, Lee JH, Choi EH, et al. SHV Hyperproduction as a Mechanism for
483 Piperacillin-Tazobactam Resistance in Extended-Spectrum Cephalosporin-Susceptible Klebsiella pneumoniae.
484 *Microb. Drug. Resist.* (Larchmont, NY). 2020;26(4):334-40.
485 65. Gilchrist CLM, Chooi Y-H. clinker & clustermap.js: automatic generation of gene cluster
486 comparison figures. *Bioinformatics*. 2021;37(16):2473-5.

**Figures**

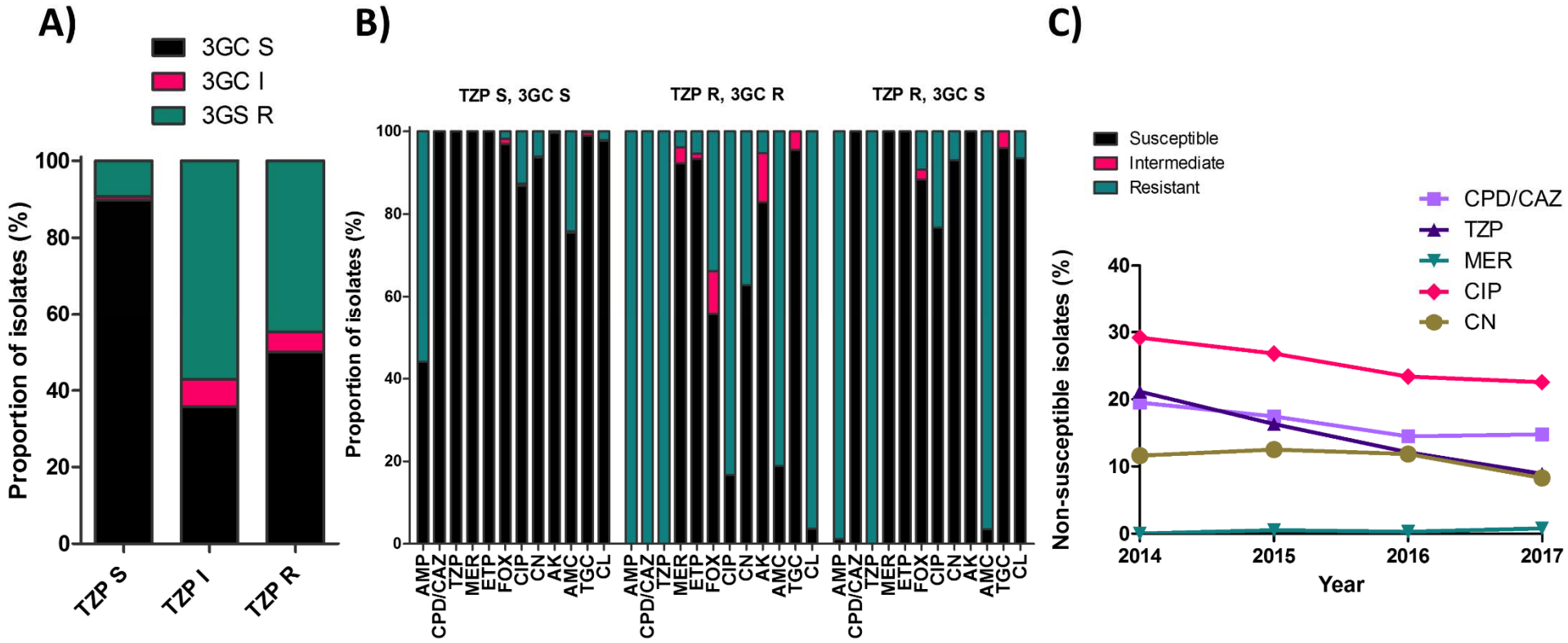


**Fig.1.(A)** Proportion of TZP susceptible (TZP S), intermediate (TZP I) and resistant (TZP R) isolates that are third generation cephalosporin susceptible (3GC S), intermediate (3GC I) and resistant (3GC R). **(B)** Antimicrobial susceptibilities of *E. coli* isolates from the RLUH, grouped by their susceptibility to piperacillin/tazobactam (TZP) and third generation cephalosporins (3GC). Susceptibility data is shown for isolates that are TZP susceptible and 3GC susceptible (TZP S, 3GC R). TZP resistant and 3GC resistant (TZP R, 3GC R), and TZP resistant and 3GC susceptible (TZP R, 3GC S), for the antibiotics

ampicillin (AMP), cefpodoxime/ceftazidime (CPD/CAZ), piperacillin/tazobactam (TZP), meropenem (MER), ertapenem (ETP), cefoxitin (FOX), ciprofloxacin (CIP), gentamycin (CN), amikacin (AK), amoxicillin/clavulanic acid (AMC), tigecycline (TGC), and chloramphenicol (CL). **(C)** Trends in non-susceptibility to CPD/CAZ, TZP, MER, CIP and CN between 2014 and 2017 at RLUH.

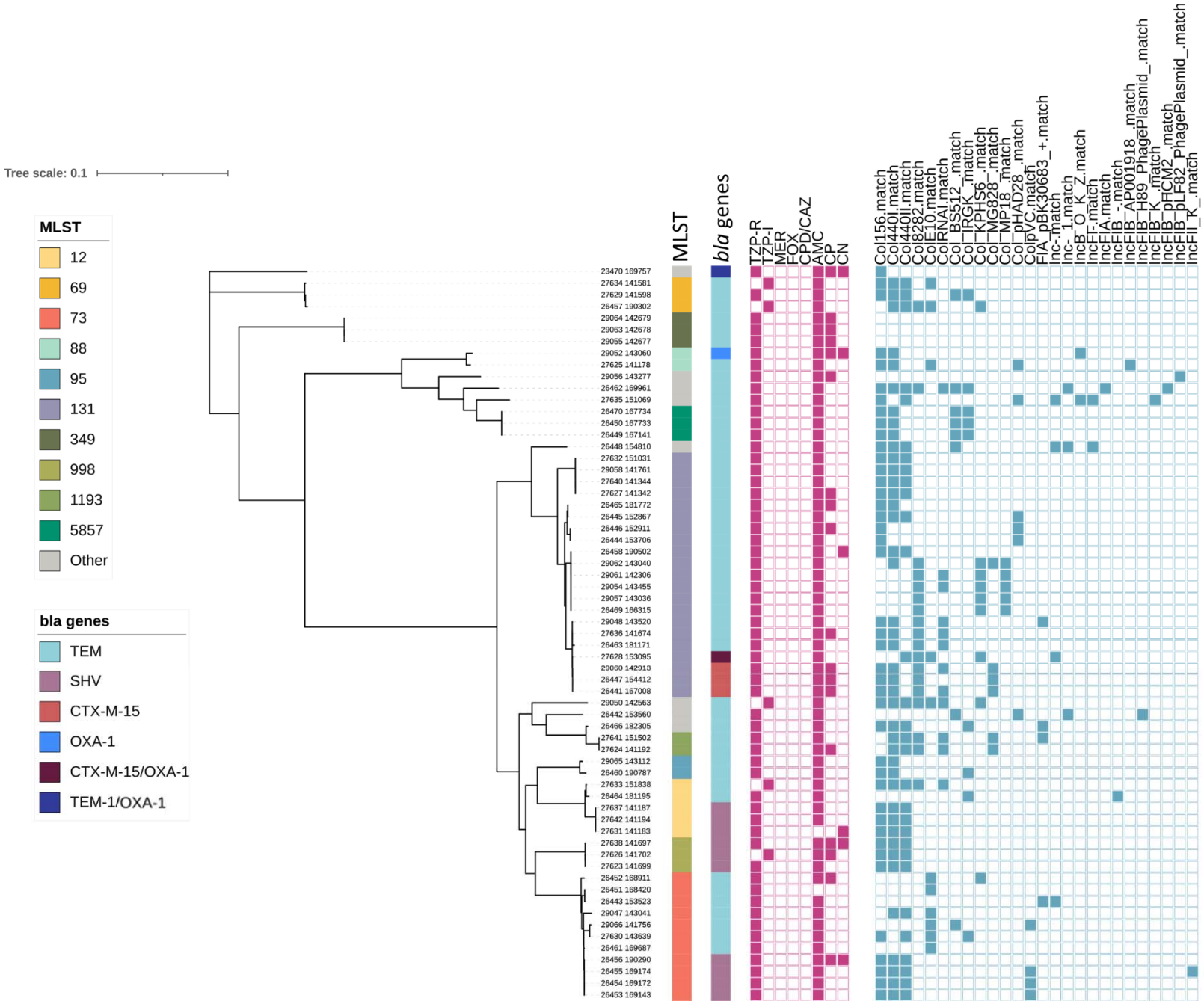
Tree scale: 0.1
MLST
12
69
73
88
95
131
349
998
1193
5857
Other
bla genes
TEM
SHV
CTX-M-15
OXA-1
CTX-M-15/OXA-1
TEM-1/OXA-1
MLST
bla genes
TZP-R
TZP-I
MER
FOX
CPD/CAZ
AMC
CP
CN
Col156.match
Col440I.match
Col440II.match
Col8282.match
ColE10.match
ColRNAI.match
Col_BS512_.match
Col_IRGK_.match
Col_KPHS6_.match
Col_MG828_.match
Col_MP18_.match
Col_pHAD28_.match
ColpVC.match
FIA_pBK30683_+.match
Inc-.match
Inc-_1.match
IncB_O_K_Z.match
IncFI-.match
IncFIA.match
IncFIB_-.match
IncFIB_AP001918_.match
IncFIB_H89_PhagePlasmid_.match
IncFIB_K_.match
IncFIB_pHCM2_.match
IncFIB_pLF82_PhagePlasmid_.match
IncFII_K_.match

**Fig.2.** Maximum likelihood phylogeny of the study isolates from RLUH. The colour strips, from left to right, show the MLST classification (MLST), β-lactamase gene carriage (*bla* genes). The heat maps show phenotypic resistance to piperacillin/tazobactam (TZP), meropenem (MER), cefoxitin (FOX), cefpodoxime/ceftazidime (CPD/CAZ), ampicillin (AMP), ciprofloxacin (CP), and gentamycin (CN), and the plasmid replicon repertoire.

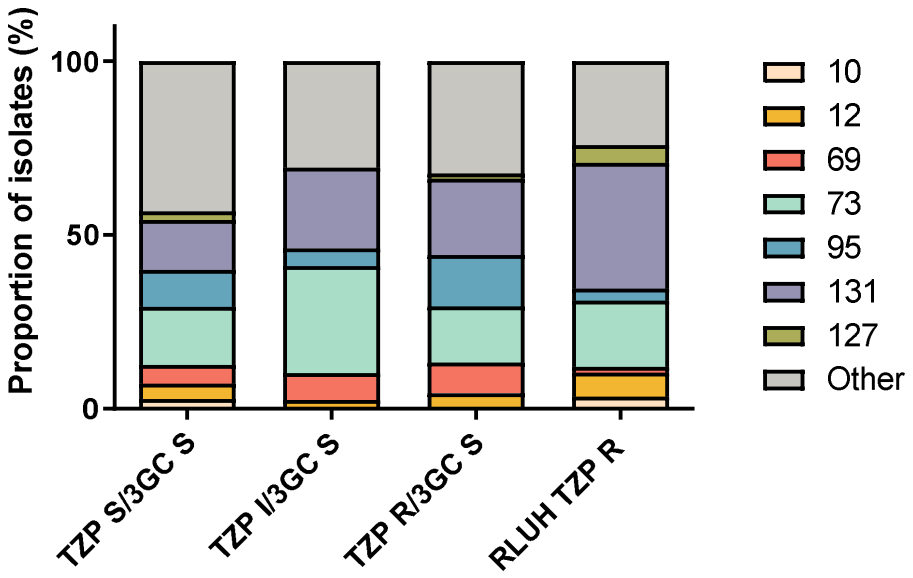


**Fig.3.** Bar chart showing the proportion of isolates belonging to common sequence types in the RLUH study isolates in comparison with those in the collection of 1509 isolates taken from a UK wide study (45).

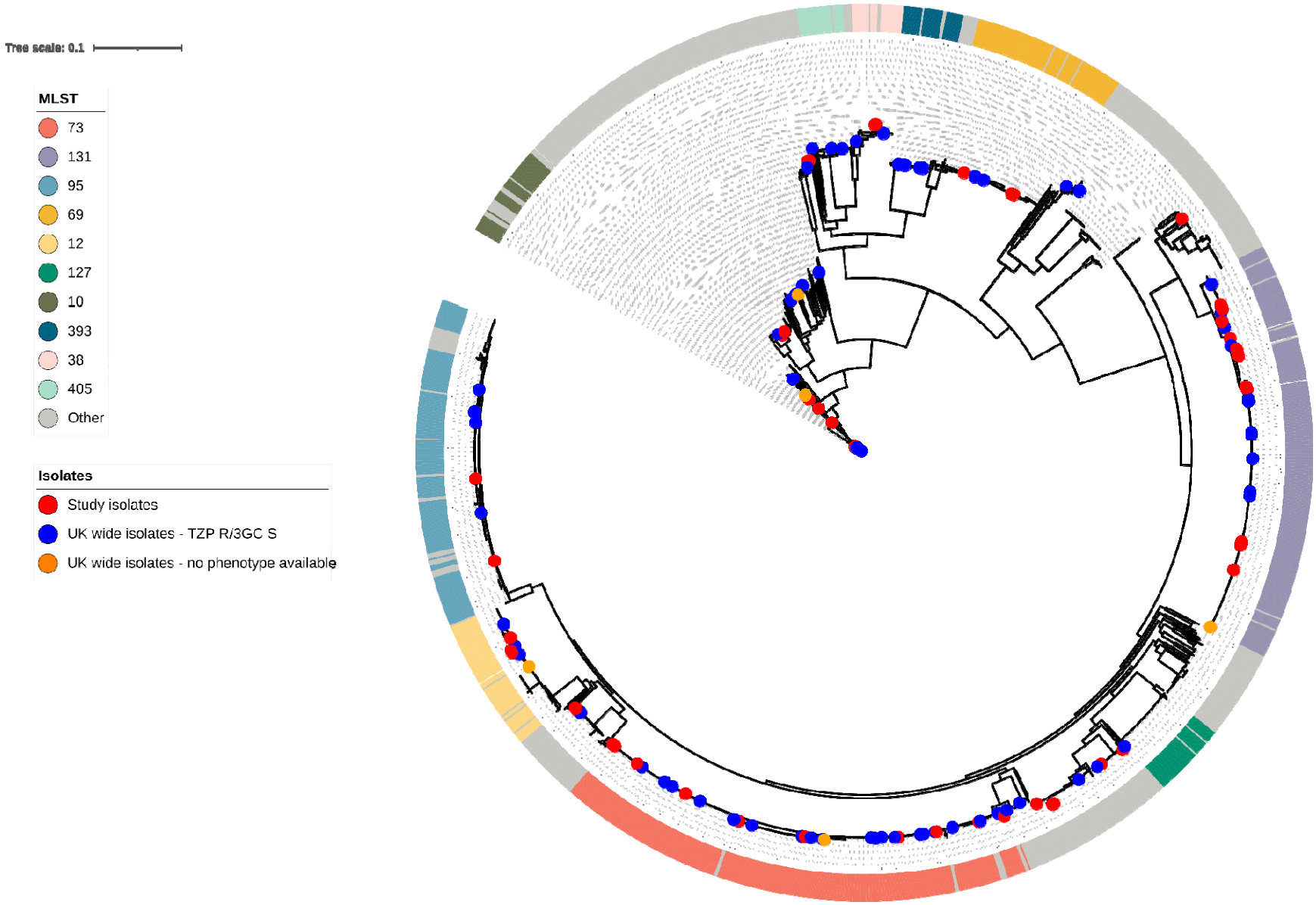
Tree scale: 0.1
MLST
73
131
95
69
12
127
10
393
38
405
Other
Isolates
Study isolates
UK wide isolates - TZP R/3GC S
UK wide isolates - no phenotype available

**Fig.4.** Circular Maximum Likelihood core genome phylogenetic tree of the 68 study isolates in combination with 1509 UK wide study isolates. The ring indicates the ten most commonly encountered STs. Dots at the terminus of branches indicate study isolates, UK wide isolates with the TZP resistant/3GC susceptible phenotype (TZP-R/3GC -S) or isolates from the UK wide collection missing sufficient phenotypic data to assign an accurate AMR phenotype.

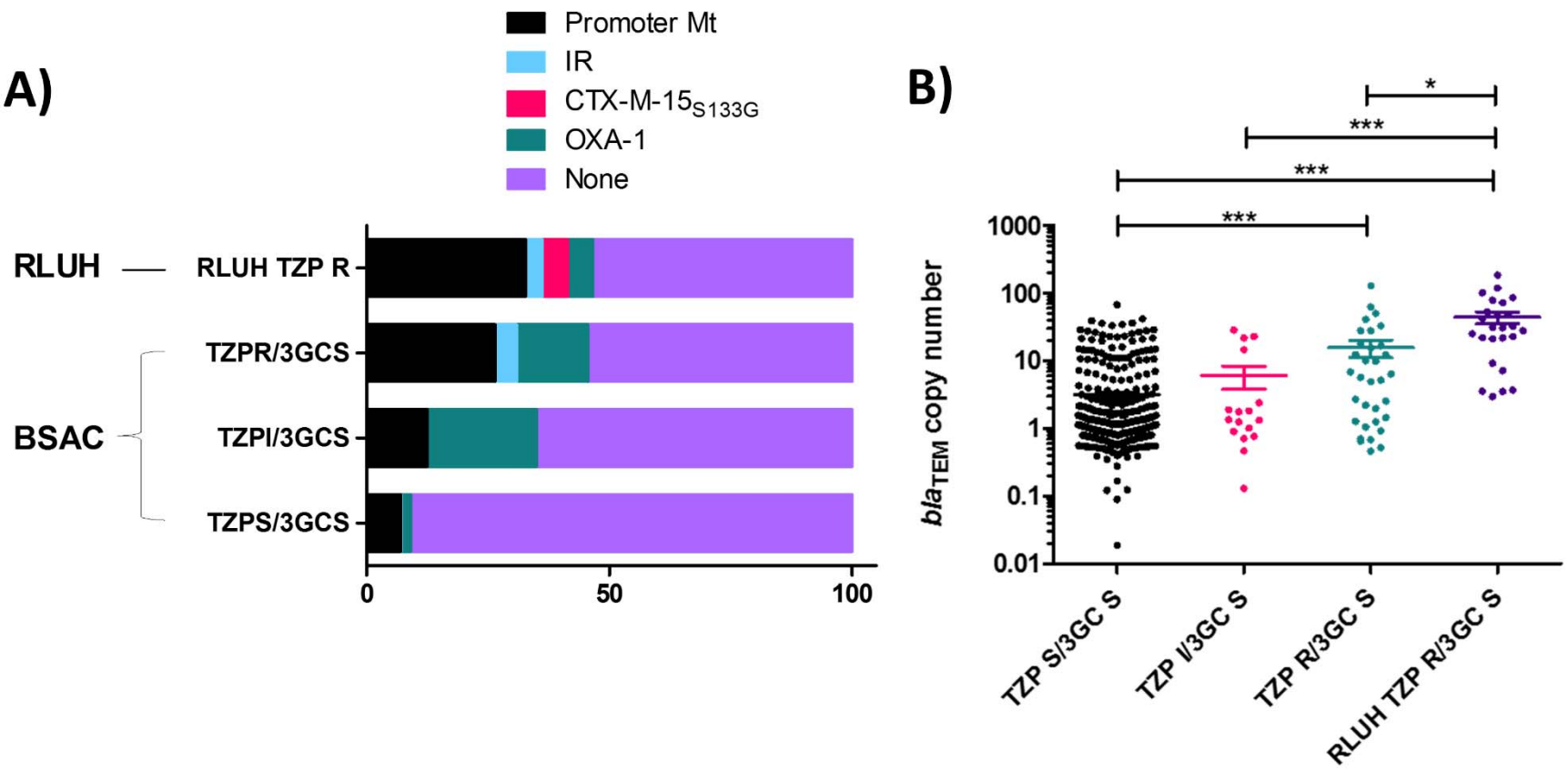
A)
Promoter Mt
IR
CTX-M-15S133G
OXA-1
None
RLUH
RLUH TZP R
BSAC
TZPR/3GCS
TZPI/3GCS
TZPS/3GCS
0
50
100
B)
*
***
***
***
1000
100
10
1
0.1
0.01
blaTEM copy number
TZP S/3GC S
TZP I/3GC S
TZP R/3GC S
RLUH TZP R/3GC S

**Fig.5.** The proportion of isolates of each phenotype identified in the RLUH and BSAC collections with identifiable putative TZP resistance mechanisms (IR; inhibitor resistance) **(A),** and the copy number of $bla_{\text{TEM-1}}$ genes found in isolates belonging to each phenotype **(B)**, with significance determined by Dunn's Multiple Comparison Test.

**Supplementary Figures**

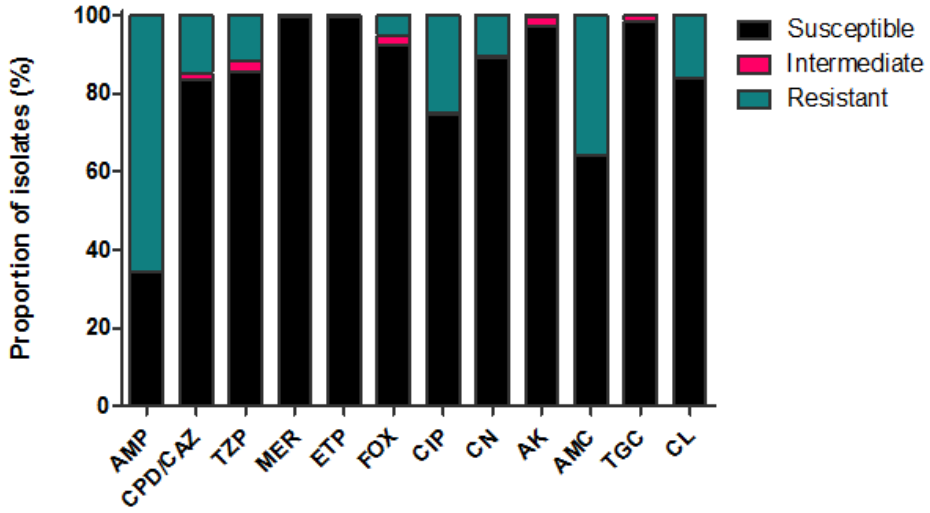


**Fig.S1.** Proportion of the total *E. coli* isolated from blood stream infections between 2014 and 2017 at RLUH that were susceptible, intermediate or resistant to ampicillin (AMP), cefpodoxime/ceftazidime (CPD/CAZ), piperacillin/tazobactam (TZP), meropenem (MER), ertapenem (ETP), cefoxitin (FOX), ciprofloxacin (CIP), gentamycin (CN), amikacin (AK), amoxicillin/clavulanic acid (AMC), tigecycline (TGC), and chloramphenicol (CL).

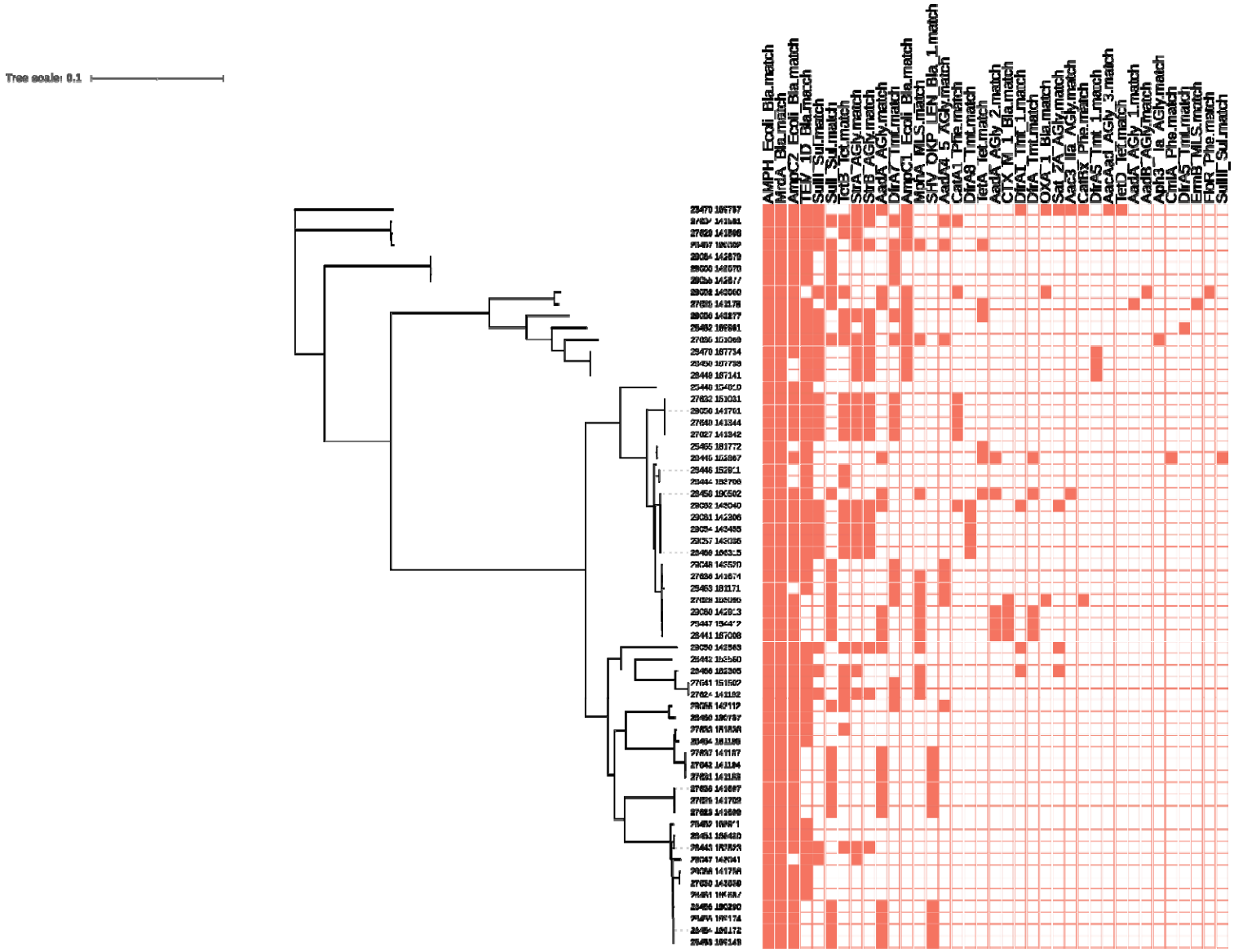


**Fig.S2** Maximum likelihood phylogeny of the study isolates from RLUH, with a heat map indicating the AMR gene repertoire.

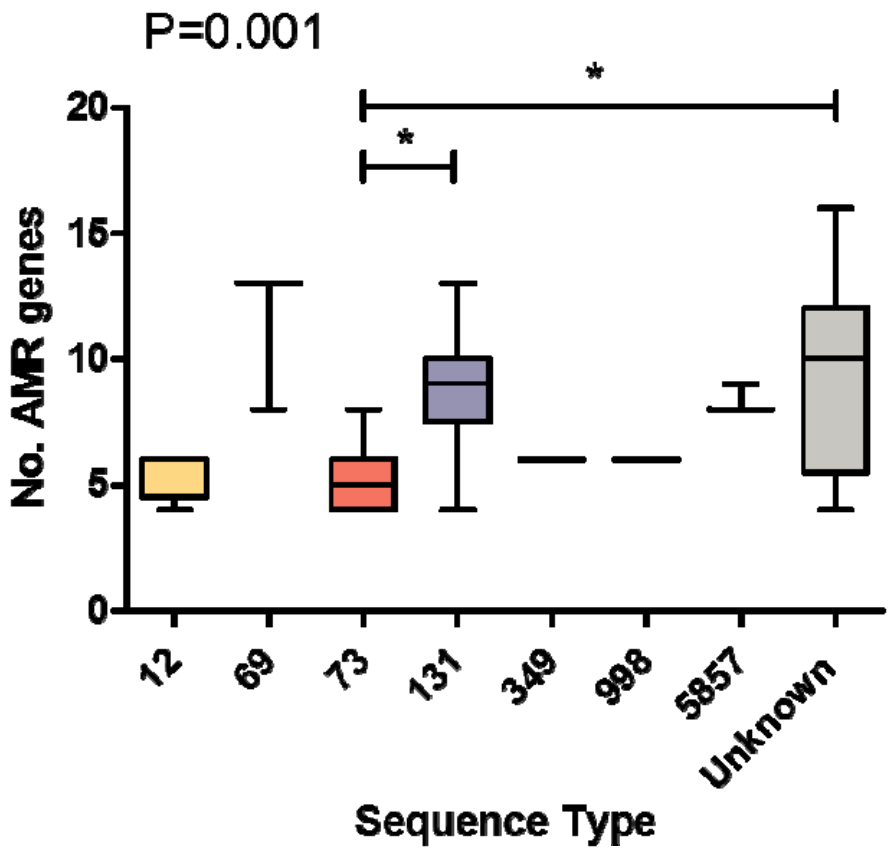


**Fig.S3** The number of AMR genes in isolates from the major sequence types encountered in the study. Whiskers show minimum and maximum values. Significance determined by Kruskal-Wallis test, * indicates a p value of <0.05.

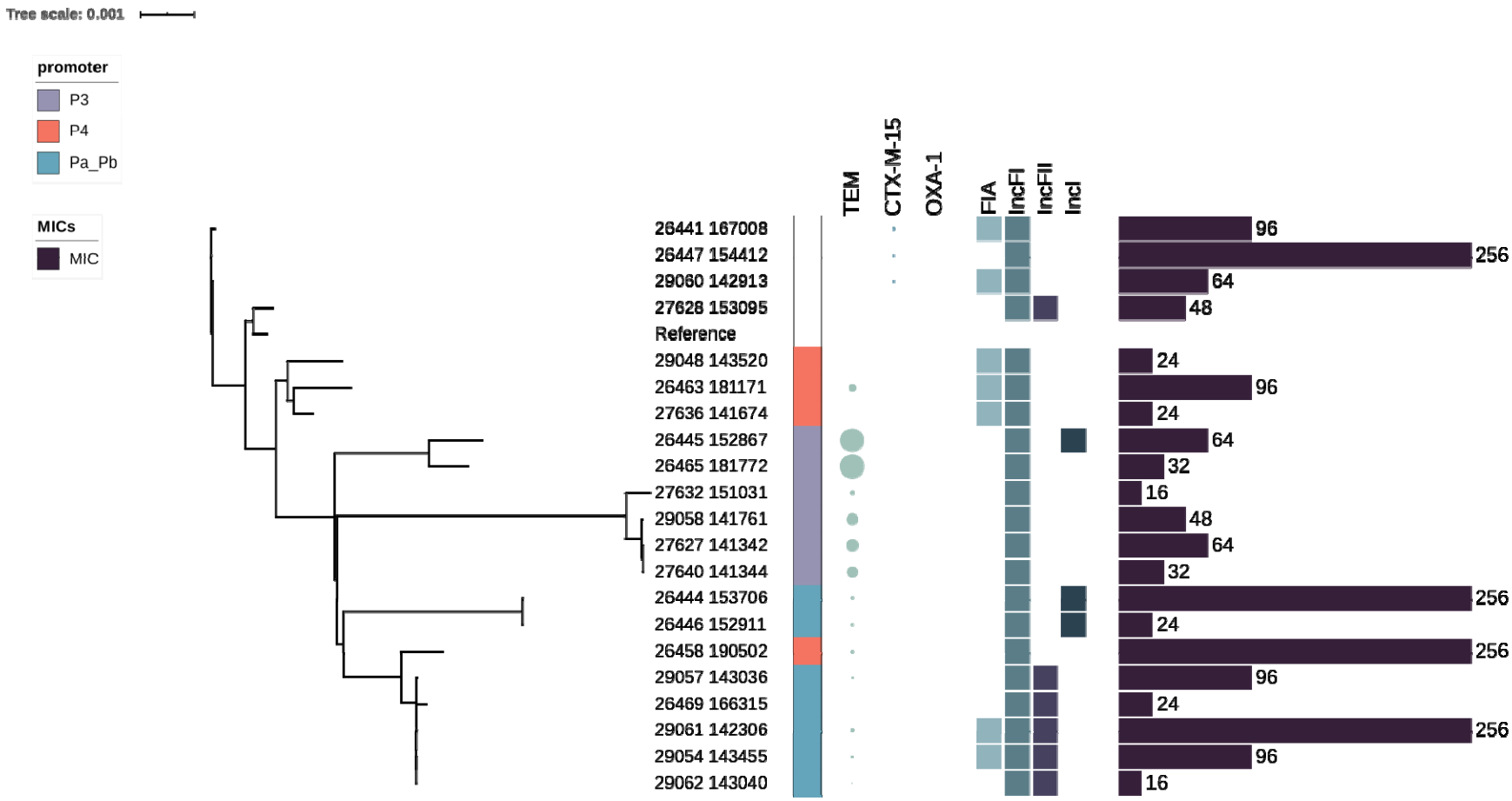


**Fig.S4.** High resolution core genome – based phylogeny of TZP resistant/3GC susceptible ST131 isolates. Indicated are promoter types, β-lactamase copy numbers (size of circle represents relative copy number), plasmid replicons, and TZP MIC.

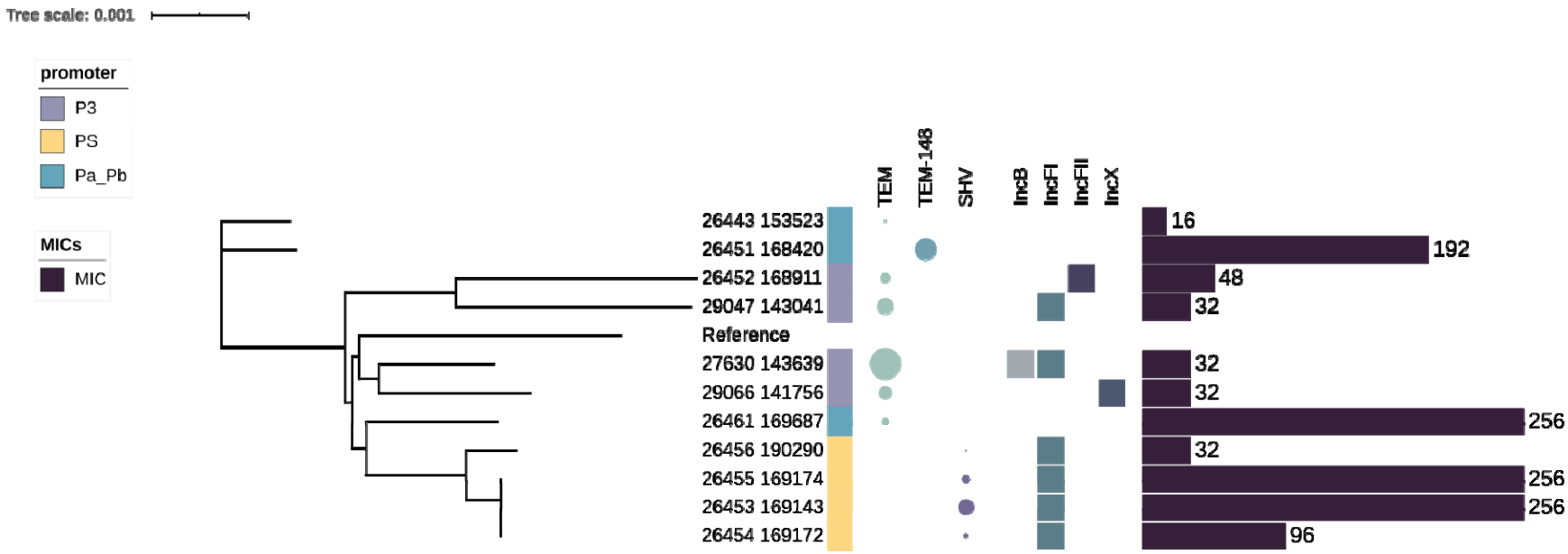


**Fig.S5.** High resolution core genome – based phylogeny of TZP resistant/3GC susceptible ST73 isolates. Indicated are promoter types, β-lactamase copy numbers (size of circle represents relative copy number), plasmid replicons, and TZP MIC.

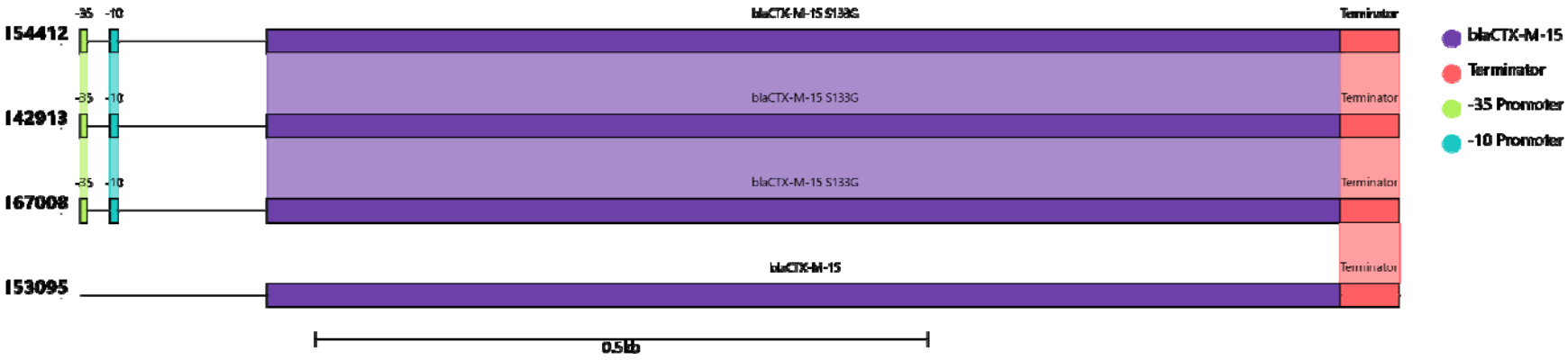


**Fig.S6**: Comparison of the promoter region, gene and terminator of blaCTX-M-15 predicted to be present in four clinical isolates from the Royal Liverpool University Hospital collection. Isolates 154412, 142913 and 167008 all contained an intact promoter and terminator with a blaCTX-M-15 containing the S133G mutation, while isolate 153095 harboured a wild type blaCTX-M-15 but lacking the promoter. Shaded regions between isolates indicate 100% identity. Figure produced using clinker (65).