



Findlay, J., Gould, V. C., North, P., Bowker, K. E., Williams, M., MacGowan, A. P., & Avison, M. B. (2020). Characterization of cefotaxime-resistant urinary *Escherichia coli* from primary care in South-West England 2017-18. *The Journal of antimicrobial chemotherapy*, 75(1), 65–71. <https://doi.org/10.1093/jac/dkz397>

Peer reviewed version

Link to published version (if available):
[10.1093/jac/dkz397](https://doi.org/10.1093/jac/dkz397)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Oxford University Press at <https://academic.oup.com/jac/article/75/1/65/5571887>. Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: <http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

1 **Characterisation of cefotaxime-resistant urinary *Escherichia coli* from primary care in**
2 **South-West England 2017-2018**

3

4 Jacqueline FINDLAY^{1*}, Virginia C. GOULD¹, Paul NORTH², Karen E. BOWKER², Martin O.
5 WILLIAMS², Alasdair P. MACGOWAN², Matthew B. AVISON¹

6

7 ¹School of Cellular & Molecular Medicine, Biomedical Sciences Building, University of Bristol,
8 University Walk, Bristol, UK.

9 ²Department of Infection Sciences, Severn Infection Partnership, Southmead Hospital, Bristol,
10 United Kingdom.

11

12

13

14

15

16

17

18

19 *Corresponding author:

20 Dr Jacqueline Findlay. Email: jacqueline.findlay@bristol.ac.uk.

21

22

23 Running heading: Cefotaxime-resistant urinary *E. coli*

24 **Abstract**

25 **Objectives:** Third-generation cephalosporin-resistant *Escherichia coli* from community-
26 acquired urinary tract infections (UTI) are increasingly reported worldwide. We sought to
27 determine and characterise the mechanisms of cefotaxime-resistance (CTX-R) employed by
28 urinary *E. coli* obtained from primary care, over 12 months, in Bristol and surrounding counties
29 in the South West of England.

30 **Methods:** Cephalexin resistant (Ceph-R) *E. coli* isolates were identified from general practice
31 (GP) referred urine samples using disc susceptibility testing. CTX-R was determined by
32 subsequent plating onto MIC breakpoint plates. β -Lactamase genes were detected by PCR.
33 WGS was performed on 225 isolates and analyses were performed using the Centre for
34 Genomic Epidemiology platform. Patient information provided by the referring GPs was
35 reviewed.

36 **Results:** Ceph-R *E. coli* (n=900) were obtained from urines from 146 GPs. Seventy-percent
37 (626/900) of isolates were CTX-R. WGS of 225 isolates identified that the most common CTX-
38 R mechanism was *bla*_{CTX-M} carriage (185/225), followed by plasmid mediated AmpCs
39 (pAmpCs) (17/225), ESBL *bla*_{SHV} variants (6/225), AmpC-hyperproduction (13/225), or a
40 combination of both *bla*_{CTX-M} and pAmpC (4/225). Forty-four STs were identified with ST131
41 representing 101/225 isolates, within which clade C2 was dominant (54/101). Ciprofloxacin-
42 resistance (CIP-R) was observed in 128/225 (56.9%) of sequenced isolates – predominantly
43 associated with fluoroquinolone-resistant clones ST131 and ST1193.

44 **Conclusions:** Most Ceph-R *E. coli*'s were CTX-R, predominantly caused by *bla*_{CTX-M} carriage.
45 The correlation between CTX-R and CIP-R was largely attributable to the high-risk pandemic
46 clones, ST131 and ST1193. Localised epidemiological data provides greater resolution than
47 regional data and can be valuable for informing treatment choices in the primary care setting.

48

49

50 Introduction

51 *Escherichia coli* that are resistant to β -lactam antibiotics, particularly to cephalosporins,
52 represent a major global public health concern. Third-generation cephalosporins (3GCs) are
53 used across the world to treat infections caused by *E. coli* (e.g. urinary tract [UTIs],
54 bloodstream [BSIs] and intra-abdominal infections) and subsequently the emergence of
55 resistance is particularly worrying.¹ Resistance to 3GCs in *E. coli* can be caused by multiple
56 mechanisms including chromosomally encoded AmpC β -lactamase hyperproduction, and
57 may involve increased efflux, and reduced outer membrane permeability, but is predominantly
58 attributed to the spread of plasmid-mediated ESBLs, (e.g. *bla*_{CTX-M}) and to a lesser extent,
59 plasmid mediated AmpCs (pAmpC, e.g. *bla*_{CMY}).² *E. coli* which harbour ESBLs are often co-
60 resistant to multiple antibiotic classes and subsequently the treatment options for such
61 infections may be limited.³

62 UTIs are the most common bacterial infection type in both primary and hospital care settings
63 in the developed world,⁴ and are associated with considerable morbidity.⁵ Previous studies of
64 community-onset UTIs (CO-UTI) in several mainland European countries found that *E. coli*
65 were the most commonly isolated uropathogen, accounting for over half of all isolates (53.3-
66 76.7%).⁶⁻⁸ The incidence of CO-UTI in the UK is difficult to determine since such infections are
67 not reportable and most are diagnosed and treated in a primary care setting, with diagnosis
68 often based solely upon patient symptoms rather than a positive urine culture. CO-UTI are
69 most often treated empirically and subsequently local epidemiological data is useful for
70 informing treatment choice. Treatment failure for CO-UTIs, particularly in immune-
71 compromised patients, increases the risk of the infection spreading to other sites including the
72 bloodstream, with grave consequences.^{9, 10}

73 *E. coli* STs belonging to phylogroups B2 (STs 73, 95 and 131) and D (ST69) have been
74 reported to be major causes of both UTIs and BSIs in the UK.¹¹ Since its initial description in
75 2008, numerous studies have shown that the multidrug-resistant pandemic clone, ST131, is a
76 major cause of UTI globally.¹²⁻¹⁴ The ST131 clonal group can be broken down by population

77 genetics into three clades based on their association with particular *fimH* types: *A/fimH41*,
78 *B/fimH22*, and *C/fimH30*.¹⁵ Clade C can be further broken down into four subclades; C1 – not
79 usually associated with ESBL carriage but typically fluoroquinolone resistant (FQ-R), C1-M27
80 and C1-nM27 – both associated with *bla*_{CTX-M-27} carriage and FQ-R, and C2 (also known as
81 H30Rx) – associated with *bla*_{CTX-M-15} carriage and FQ-R.^{15, 16} Studies have suggested that the
82 global dominance of ESBL-positive ST131 is, in part, due to its increased virulence potential
83 over its non-ST131 ESBL-positive counterparts.^{17, 18}

84 Although cephalosporin use for the treatment of UTIs is not typical in the UK, they may be
85 used where resistance to first and second-line treatment exists. This may select cephalosporin
86 resistant UTIs, and if an invasive infection occurs, empiric therapy with a 3GC is likely to fail.
87 This study sought to use WGS to characterise the population structure and determine the
88 mechanisms of resistance to the 3GC cefotaxime (CTX-R) employed by urinary *E. coli* isolates
89 referred from general practices (GPs) in Bristol and surrounding counties in the South-West
90 of England serving a population of approximately 1.2 million people.

91

92 **Materials and Methods**

93 **Bacterial isolates, identification and susceptibility testing**

94 Cephalexin-resistant (Ceph-R) urinary *E. coli* isolates were obtained from routine urine
95 microbiology at Severn Infection Partnership Southmead Hospital. Urine samples were
96 submitted between Sept 2017 and Aug 2018, from 146 GPs located throughout Bristol and
97 including coverage in Gloucestershire, Somerset and Wiltshire.

98 Bacterial identification was carried out using BD™ CHROMagar™ Orientation Medium
99 chromogenic agar (BD, GmbH, Heidelberg, Germany).

100 Antibiotic susceptibilities were performed by disc testing or, in the case of colistin, by broth
101 microdilution and interpreted according to EUCAST guidelines.¹⁹ A single colony from Ceph-

102 R isolates was subcultured onto TBX agar plates (Sigma-Aldrich, Dorset, UK) containing 2
103 mg/L CTX and isolates that were positive for growth were deemed CTX-R, and taken forward
104 for further molecular testing.

105 **Screening for β -lactamase genes**

106 Two multiplex PCRs were performed to screen for β -lactamase genes. The first to detect
107 *bla*_{CTX-M} groups as previously described,²⁰ and the second to detect the following additional β -
108 lactamase genes; *bla*_{CMY-2} type, *bla*_{DHA}, *bla*_{SHV}, *bla*_{TEM}, *bla*_{OXA-1}, using the primers listed in Table
109 S1.

110 **WGS and analyses**

111 WGS was performed by MicrobesNG (<https://microbesng.uk/>) on a HiSeq 2500 instrument
112 (Illumina, San Diego, CA, USA) using 2x250 bp paired end reads. Reads were trimmed using
113 Trimmomatic,²¹ assembled into contigs using SPAdes²² 3.13.0
114 (<http://cab.spbu.ru/software/spades/>) and contigs were annotated using Prokka.²³ Resistance
115 genes, plasmid replicon types, sequence types and *fim* types were assigned using the
116 ResFinder,²⁴ PlasmidFinder,²⁵ MLST²⁶ 2.0 and FimTyper on the Center for Genomic
117 Epidemiology (<http://www.genomicepidemiology.org/>) platform.

118 ST131 clades were identified by resistance gene carriage and *fimH* type, and in the case of
119 clade C1-M27, by the presence of the prophage region M27PP1 (CP006632)¹⁶ through
120 sequence alignment using progressive Mauve alignment software.²⁷

121 MLST and resistance gene data were analysed to produce a minimum spanning tree using
122 Bionumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

123 Plasmid pUB_DHA-1 assembled onto a single contig. The overlap of contig ends was
124 confirmed by PCR, and the plasmid sequence was submitted to GenBank with accession
125 number MK048477. Reads were mapped using Geneious Prime 2019.1.3
126 (<https://www.geneious.com>).

127 **Plasmid Transformation**

128 Transformation of plasmid extractions from isolates encoding *mcr-1* and *bla*_{OXA-244} were
129 attempted by electroporation using *E. coli* DH5 alpha as a recipient. Transformants were
130 selected, respectively, on LB agar containing 0.5 mg/L colistin, or containing 100 mg/L
131 ampicillin with a 10 µg ertapenem disc being placed on the agar surface (Oxoid Ltd,
132 Basingstoke, UK). Transformants were confirmed by PCR (Table S1).

133 **Analysis of patient demographic information**

134 Limited, non-identifiable patient information was obtained from the request forms sent with
135 submissions from referring GPs.

136

137 **Results and Discussion**

138 **Patient demographics and antimicrobial susceptibilities**

139 *E. coli* is cultured from approximately two-thirds of all bacterium-positive GP-submitted urine
140 samples processed by the Southmead Hospital laboratory, totalling ~36,000 isolates per year
141 . Trimethoprim and nitrofurantoin resistance is observed in ~35% and 2%, and Ceph-R in ~8%
142 of these *E. coli*, respectively. If the *E. coli* is Ceph-R, trimethoprim and nitrofurantoin resistance
143 rates increase to ~67% and ~7%, respectively. 3GC resistance is seen in ~72% of Ceph-R *E.*
144 *coli*.

145 Nine hundred Ceph-R urinary *E. coli* isolates were collected during the period of our study.
146 Following deduplication by patient, isolates were obtained from 836 patients. Most isolates
147 were obtained from female patients (669/836; 80.0%) and the mean patient age was 62.4
148 years (median = 69 years). Almost sixty-nine percent (576/836) were CTX-R, which matches
149 phenotypic laboratory data, confirming that this is a representative sample. Most CTX-R
150 isolates were again from females (465/576; 80.7%) and the mean patient age was 62.5 years
151 (median = 69 years).

152

153 **β-Lactamase genes of interest detected by PCR in CTX-R isolates**

154 Table 1 indicates the number of CTX-R isolates carrying each β-lactamase gene of interest
155 (GOI): *bla*_{CTX-Ms}, *bla*_{CMY}, *bla*_{DHA} or *bla*_{SHV}. Of these, *bla*_{CTX-Ms} were by far the most prevalent,
156 found in 571/626 (91.2%) of isolates. Within these, *bla*_{CTX-M-G1} were most common (421/626)
157 followed by *bla*_{CTX-M-G9} (149/626) and *bla*_{CTX-M-G8} (one isolate). A previous study performed
158 across four primary care trusts in England in 2013-14 estimated the carriage of CTX-M-
159 producing Enterobacteriaceae in the faeces of healthy adults at 7.3% (range of 4.9-16.0%),
160 over 95% of which were *E. coli*.²⁸ Based on our data above, 5.3% of GP-submitted urinary *E.*
161 *coli* were CTX-M positive.

162 pAmpCs *bla*_{CMY} and *bla*_{DHA} were found in 13 (3 alongside *bla*_{CTX-M-G1}) and 17 (4 alongside
163 *bla*_{CTX-M-G1}) isolates respectively. *bla*_{SHV} was found in 11 (3 alongside *bla*_{CTX-M-G1}) isolates and
164 the remaining 24 isolates harboured none of the GOIs as detected by PCR.

165 **Whole Genome Sequencing (WGS) analyses**

166 **GOI variants and STs**

167 Two-hundred and twenty-five isolates, chosen to be representative of resistance gene
168 carriage (as previously determined by PCR) and patient demographics (age, sex) obtained
169 throughout the entire study period, were selected for WGS. Within these, 44 sequence types
170 (STs) were identified, with numbers of isolates ranging from 1 to 101 representatives. ST131
171 was dominant (n=101), followed by STs 69 (n=15), 73 (n=15), 38 (n=13), 1193 (n=11), and 10
172 (n=8). The remaining 38 STs had 1 to 4 representative isolates (Figure 1).

173 CTX-R GOIs were identified in all but thirteen isolates (212/225; 94.2%). Eighty-four percent
174 (189/225) of isolates harboured one of seven *bla*_{CTX-M} gene variants (Table 2). Carriage of
175 *bla*_{CTX-M-15} was the most common CTX-R mechanism identified (118/189) followed by *bla*_{CTX-M-}
176 ₂₇ (44/189) and *bla*_{CTX-M-14} (10/189). Amongst the non-CTX-M GOIs, four *bla*_{CMY} variants were

177 identified; *bla*_{CMY-2} (n=7), *bla*_{CMY-4} (n=1), *bla*_{CMY-42} (n=2) and *bla*_{CMY-60} (n=3; all three being co-
178 carried alongside *bla*_{CTX-M-15}), as well as *bla*_{DHA-1} (n=8; one alongside *bla*_{CTX-M-15}) and *bla*_{SHV-12}
179 (n=6). The narrow spectrum β-lactamases *bla*_{OXA-1}, *bla*_{TEM-1} and inhibitor-resistant variant
180 *bla*_{TEM-33} were found in 53, 82, and one isolate respectively.

181 **AmpC-hyperproducing isolates**

182 All thirteen (5.8%) sequenced isolates, where no CTX-R GOI could be identified, were
183 presumed to be chromosomal AmpC β-lactamase hyperproducers because they carry
184 mutations within the *ampC* promoter/attenuator region previously seen in confirmed AmpC
185 hyperproducers (Table 3).^{29, 30} These represented nine different STs, each having one
186 representative, with the exceptions of the STs 75 and 200 of which there were three
187 representatives each (Table S2). This indicates a lack of dominant clones in AmpC
188 hyperproducers identified in this study. If we go on to assume that the 24 isolates negative for
189 GOIs by PCR are AmpC-hyperproducers, as was found with the thirteen representative
190 sequenced isolates, then 3.8% of the isolates in this study could be classed as AmpC-
191 hyperproducers. Additionally, one *bla*_{CTX-M-15}-positive isolate was found to also harbour *ampC*
192 promoter changes associated with hyperproduction.

193 **Characterisation of ST131 Isolates**

194 **ESBLs and clades**

195 One hundred and one ST131 isolates harboured the following CTX-R mechanisms/alleles;
196 *bla*_{CTX-M-1} (n=1), *bla*_{CTX-M-14} (n=4), *bla*_{CTX-M-15} (n=61), *bla*_{CTX-M-15}/*bla*_{CMY-60} (n=1), *bla*_{CTX-M-27}
197 (n=33). The isolates were broken down into their respective clades (Table 4); ST131-C2 was
198 dominant (54/101; 53.5%) followed by C1-M27 (M27) (23/101; 22.8%), A (11/101; 10.9%), C1-
199 nM27 (5/101; 5.0%), one clade B, and seven isolates were unclassified. Eighty-eight percent
200 (89/101) of isolates, and notably all clade C2 isolates, were CIP-R as is a typical characteristic
201 of this lineage. Two non-ST131 members of the ST131 complex, both of which were *bla*_{CTX-M-15}-
202 positive ST8313 isolates (a *fumC* single locus variant (SLV) of ST131), also harboured the

203 same chromosomal FQ-R associated mutations in *gyrA*, *parC* and *parE* as are associated with
204 ST131/C2 – suggesting this ST may be ST131/C2 derived. Previous studies have highlighted
205 the dominance of ST131 and particularly the clade C2/H30Rx on a worldwide scale.^{13, 14} Since
206 its initial description in 2008 in isolates from 3 continents,^{12, 13} ST131 has been reported across
207 all inhabited continents.¹⁵ The recently described C1 subclade, C1-M27, as characterised by
208 the presence of a 11,894 bp prophage-like genomic island M27PP1, was initially described in
209 Japan in 2016 and has been reported in countries in at least three continents: Europe, Asia
210 and North America, so far.¹⁶ The presence of C1-M27 isolates in this study indicates the
211 expansion of this particular ST131 sublineage into the UK, similarly to that which has been
212 reported from countries in mainland Europe.³¹ There was no geographical clustering of C1-
213 M27 positive isolates within our study region.

214 **Virotypes**

215 ST131 has been reported in previous studies to be a highly virulent clone, exhibiting lethality
216 in mouse sepsis models.^{18, 32} Virotypes of all 101 ST131 isolates were determined as
217 previously described.^{18, 33} Virotype C was most common, represented by 38/101 (37.6%) of
218 ST131 isolates and predominantly associated with *bla*_{CTX-M-27} (28/38 isolates) across clades A
219 (n=3), C1-M27 (n=22), C1-nM27 (n=4), and one isolate belonged to an unclassified clade. All
220 *bla*_{CTX-M-27}-positive isolates belonged to virotype C, with the exception of one isolate for which
221 a virotype could not be assigned. The association between virotype C and *bla*_{CTX-M-27} carriage
222 is in agreement with a recent study conducted in France.³⁴ Twenty-five isolates belonged to
223 virotype A, all of which except one harboured *bla*_{CTX-M-15}. The remaining 38 isolates belonged
224 to either virotype B (n=1), D (n=1), G (n=8), or were unknown virotypes (n=28).

225 **Genetic context of ESBLs/pAmpC genes**

226 Despite the limitations of short read sequencing, by examining the contigs on which GOs
227 were located, we were able to determine the chromosomal or plasmid environments of
228 ESBLs/pAmpC genes in 85/225 isolates. Forty *bla*_{CTX-M} genes, of variants *bla*_{CTX-M-14} (n=3) and

229 *bla*_{CTX-M-15} (n=37), were found to be located on the chromosome. These were found in 11 STs
230 with STs 131 (n=11) and 73 (n=10) being the most represented. Whilst the *bla*_{CTX-M} genetic
231 environments were diverse in ST131, in ST73, 9/10 isolates harboured the gene in the same
232 genomic location, suggesting a high degree of clonality within this ST. Chromosomally
233 encoded *bla*_{CTX-M} genes have previously been reported in 12.9% of human isolates in study of
234 ESBL *E. coli* performed in Germany, The Netherlands and the UK.³⁵ The remaining GOIs were
235 found to be located within a relatively diverse range of plasmids and across multiple STs.
236 Interestingly all six DHA-1-harboring isolates were found to harbour a similar IncI1 plasmid
237 which was sequenced to closure during this study, pUB_DHA-1. Read mapping analyses
238 showed that all six isolates exhibited 95-100% coverage and 98-100% identity against
239 pUB_DHA-1.

240 **Other important resistance genes found by WGS**

241 Interestingly one ST69 CMY-2-producing isolate was also found to harbour *mcr-1*.
242 Susceptibility testing, performed by broth microdilution, revealed that the MIC of colistin
243 against this isolate is 8 mg/L and so it is colistin resistant. Attempts at transformation of *E. coli*
244 DH5 alpha using plasmid DNA extracted from this isolate were successful indicating that *mcr-*
245 *1* is plasmid encoded. Analysis of the genetic environment of *mcr-1* found that it is encoded
246 on an IncI2 plasmid of approximately 62 kb and it lacks the upstream IS*ApI1* element that was
247 described in the initial discovery of *mcr-1* in China.³⁶ The plasmid itself does not encode any
248 additional resistance genes and when subjected to NCBI BLAST analysis exhibited ~96%
249 similarity to *mcr-1* encoding plasmids found in both China (pHNGDF93; Genbank Accession
250 Number MF978388) and Taiwan (p5CRE51-MCR-1; Genbank Accession Number CP021176)
251 from animal and human origins, respectively. Since initial reports of its discovery in 2015,³⁶
252 *mcr-1* has been reported worldwide in clinical *E. coli* isolates although remains relatively rare.
253 Another isolate, a *bla*_{CTX-M-14}-positive ST38, also encoded the *bla*_{OXA-48}-like carbapenemase
254 gene *bla*_{OXA-244}.³⁷ Transformation attempts using plasmid DNA from this isolate were
255 unsuccessful and so it was concluded that *bla*_{OXA-244} is likely to be chromosomally encoded.

256 Disc susceptibility testing showed that this isolate was resistant to ertapenem but susceptible
257 to both imipenem and meropenem. The presence of the chromosomally-encoded OXA-48-like
258 carbapenemase *bla*_{OXA-244}, confirms the observations of a previous study, where OXA-48-like
259 genes were shown to have become embedded in the ST38 chromosome.³⁸ ST38 is the most
260 frequent ST associated with OXA-48-like enzymes in the UK.³⁸

261 **Conclusions**

262 Resistance to 3GCs and FQs in *E. coli* is of increasing concern due to the importance of these
263 classes of drugs for the treatment of serious infections. Increasing resistance to 3GCs puts
264 increased pressure and reliance on carbapenems, often referred to as the ‘antibiotics of last
265 resort’ for serious MDR Gram-negative infections and subsequently the surveillance of these
266 resistances is essential. As observed in this study and in line with previous reports globally,²
267 the dissemination of successful clones, and/or ST lineages (clades), is a major cause of CTX-
268 R in urinary isolates from primary care in South West England.

269 *bla*_{CTX-M}-positive *E. coli* have been observed in our study region since initial reports in 2000.³⁹
270 The prevalence of *bla*_{CTX-M} genes observed in the CTX-R isolates in this study is reflective of
271 the observed rates of CTX-M *E. coli* faecal carriage in healthy adults in the UK, as has been
272 quantified in previous studies.^{28, 40}

273 The correlation between CIP-R and CTX-R highlighted here can be largely attributed to the
274 dominance of successful clones/clades, namely ST131 and ST1193; the majority of both
275 harbour chromosomal FQ-R mutations. Through WGS of a subset of isolates we have shown
276 that ST131 clade C2 is dominant and that the recently described ST131 subclade, C1-M27, is
277 also prevalent despite not previously being described in the UK.

278 This study is the first analysis of CTX-R *E. coli* causing CO-UTI performed in a relatively
279 localised area in South West England and could be useful for informing patient treatment,
280 alongside resistance information generated in the hospital laboratory, to provide essential data
281 for comparison purposes to other areas, both within and outwith the UK.

282

283 **Acknowledgements**

284 Genome sequencing was provided by MicrobesNG (<http://www.microbesng.uk>), which is
285 supported by the BBSRC (grant number BB/L024209/1).

286 **Funding**

287 This work was funded by grant NE/N01961X/1 to M.B.A. and A.P.MacG. from the Antimicrobial
288 Resistance Cross Council Initiative supported by the seven United Kingdom research
289 councils.

290 **Transparency declaration**

291 None to declare.

292

293 **Supplementary data**

294 Tables S1 and S2 are available as supplementary data.

295

296

297 **References**

298

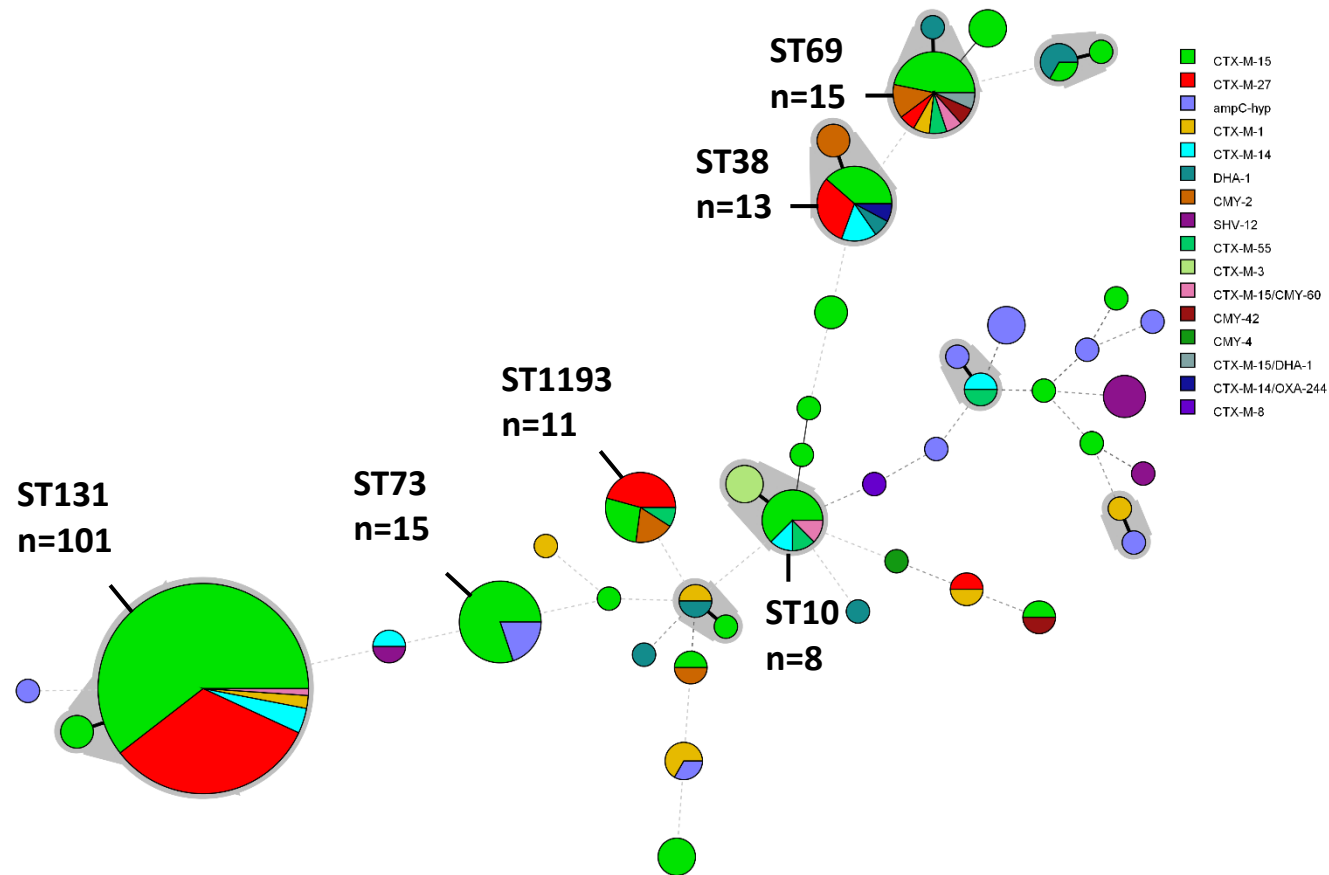
- 299 1. Public Health England. English surveillance programme for antimicrobial utilisation and
300 resistance (ESPAUR). 2018.
301 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf)
302 [759975/ESPAUR_2018_report.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/759975/ESPAUR_2018_report.pdf).
- 303 2. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M beta-lactamases: temporal
304 and geographical shifts in genotype. *J Antimicrob Chemother* 2017; **72**: 2145-55.
- 305 3. Morosini MI, Garcia-Castillo M, Coque TM *et al*. Antibiotic coresistance in extended-
306 spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline.
307 *Antimicrob Agents Chemother* 2006; **50**: 2695-9.
- 308 4. Nicolle LE. Epidemiology of urinary tract infections. *Clin Microb News* 2002; **24**: 135-40.
- 309 5. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic
310 costs. *Am J Med* 2002; **113 Suppl 1A**: 5s-13s.
- 311 6. Kahlmeter G, Eco.Sens. An international survey of the antimicrobial susceptibility of
312 pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob*
313 *Chemother* 2003; **51**: 69-76.
- 314 7. Cordoba G, Holm A, Hansen F *et al*. Prevalence of antimicrobial resistant *Escherichia coli*
315 from patients with suspected urinary tract infection in primary care, Denmark. *BMC Infect Dis* 2017;
316 **17**: 670.
- 317 8. Schito GC, Naber KG, Botto H *et al*. The ARESC study: an international survey on the
318 antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J*
319 *Antimicrob Agents* 2009; **34**: 407-13.
- 320 9. Song JAW, Berridge D, Akbari A, *et al*. Risk factors for *Escherichia coli* bacteraemia: a
321 population-based case-control study. *Lancet; Meeting Abstracts* 2017; **390**: S85.
- 322 10. Lishman H, Costelloe C, Hopkins S *et al*. Exploring the relationship between primary care
323 antibiotic prescribing for urinary tract infections, *Escherichia coli* bacteraemia incidence and
324 antimicrobial resistance: an ecological study. *Int J Antimicrob Agents* 2018; **52**: 790-8.
- 325 11. Doumith M, Day M, Ciesielczuk H *et al*. Rapid identification of major *Escherichia coli*
326 sequence types causing urinary tract and bloodstream infections. *J Clin Microbiol* 2015; **53**: 160-6.
- 327 12. Coque TM, Novais A, Carattoli A *et al*. Dissemination of clonally related *Escherichia coli*
328 strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* 2008; **14**: 195-
329 200.
- 330 13. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al*. Intercontinental emergence of
331 *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273-81.
- 332 14. Petty NK, Ben Zakour NL, Stanton-Cook M *et al*. Global dissemination of a multidrug resistant
333 *Escherichia coli* clone. *Proc Nat Acad Sci USA* 2014; **111**: 5694-9.
- 334 15. Pitout JD, DeVinney R. *Escherichia coli* ST131: a multidrug-resistant clone primed for global
335 domination. *F1000 Res* 2017; **6**.
- 336 16. Matsumura Y, Pitout JD, Gomi R *et al*. Global *Escherichia coli* Sequence Type 131 Clade with
337 blaCTX-M-27 Gene. *Emerg Infect Dis* 2016; **22**: 1900-7.
- 338 17. Banerjee R, Robicsek A, Kuskowski MA *et al*. Molecular epidemiology of *Escherichia coli*
339 sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-beta-lactamase-
340 positive and -negative *E. coli* clinical isolates from the Chicago Region, 2007 to 2010. *Antimicrob*
341 *Agents Chemother* 2013; **57**: 6385-8.
- 342 18. Mora A, Dahbi G, Lopez C *et al*. Virulence patterns in a murine sepsis model of ST131
343 *Escherichia coli* clinical isolates belonging to serotypes O25b:H4 and O16:H5 are associated to
344 specific virotypes. *PLoS One* 2014; **9**: e87025.
- 345 19. European Committee of Antimicrobial Susceptibility Testing. Breakpoint tables for
346 interpretation of MICs and zone diameters. Version 8.1.

347 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf)
348 [Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf).

- 349 20. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding
350 CTX-M extended-spectrum (beta)-lactamases. *J Antimicrob Chemother* 2006; **57**: 154-5.
- 351 21. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data.
352 *Bioinformatics* 2014; **30**: 2114-20.
- 353 22. Bankevich A, Nurk S, Antipov D *et al*. SPAdes: a new genome assembly algorithm and its
354 applications to single-cell sequencing. *J Comp Biol* 2012; **19**: 455-77.
- 355 23. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014; **30**: 2068-9.
- 356 24. Zankari E, Hasman H, Cosentino S *et al*. Identification of acquired antimicrobial resistance
357 genes. *J Antimicrob Chemother* 2012; **67**: 2640-4.
- 358 25. Carattoli A, Zankari E, Garcia-Fernandez A *et al*. In silico detection and typing of plasmids
359 using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;
360 **58**: 3895-903.
- 361 26. Wirth T, Falush D, Lan R *et al*. Sex and virulence in *Escherichia coli*: an evolutionary
362 perspective. *Mol Microbiol* 2006; **60**: 1136-51.
- 363 27. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain,
364 loss and rearrangement. *PLoS One* 2010; **5**: e11147.
- 365 28. McNulty CAM, Lecky DM, Xu-McCrae L *et al*. CTX-M ESBL-producing Enterobacteriaceae:
366 estimated prevalence in adults in England in 2014. *J Antimicrob Chemother* 2018; **73**: 1368-88.
- 367 29. Caroff N, Espaze E, Gautreau D *et al*. Analysis of the effects of -42 and -32 ampC promoter
368 mutations in clinical isolates of *Escherichia coli* hyperproducing ampC. *J Antimicrob Chemother* 2000;
369 **45**: 783-8.
- 370 30. Peter-Getzlaff S, Polsfuss S, Poledica M *et al*. Detection of AmpC beta-lactamase in
371 *Escherichia coli*: comparison of three phenotypic confirmation assays and genetic analysis. *J Clin*
372 *Microbiol* 2011; **49**: 2924-32.
- 373 31. Merino I, Hernandez-Garcia M, Turrientes MC *et al*. Emergence of ESBL-producing
374 *Escherichia coli* ST131-C1-M27 clade colonizing patients in Europe. *J Antimicrob Chemother* 2018; **73**:
375 2973-80.
- 376 32. Clermont O, Lavollay M, Vimont S *et al*. The CTX-M-15-producing *Escherichia coli* diffusing
377 clone belongs to a highly virulent B2 phylogenetic subgroup. *J Antimicrob Chemother* 2008; **61**: 1024-
378 8.
- 379 33. Barrios-Villa E, Cortes-Cortes G, Lozano-Zarain P *et al*. Adherent/invasive *Escherichia coli*
380 (AIEC) isolates from asymptomatic people: new *E. coli* ST131 O25:H4/H30-Rx virotypes. *Ann Clin*
381 *Microbiol Antimicrob* 2018; **17**: 42.
- 382 34. Birgy A, Levy C, Nicolas-Chanoine MH *et al*. Emergence and dominance of *E. coli* ST131 CTX-
383 M-27 in a community paediatric cohort study: independent host factors and bacterial genetic
384 determinants. *Antimicrob Agents Chemother* 2019; 63:e00382-19.
- 385 35. Rodriguez I, Thomas K, Van Essen A *et al*. Chromosomal location of *bla*_{CTX-M} genes in clinical
386 isolates of *Escherichia coli* from Germany, The Netherlands and the UK. *Int J Antimicrob Agents* 2014;
387 **43**: 553-7.
- 388 36. Liu YY, Wang Y, Walsh TR *et al*. Emergence of plasmid-mediated colistin resistance
389 mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological
390 study. *Lancet Infect Dis* 2016; **16**: 161-8.
- 391 37. Oteo J, Hernandez JM, Espasa M *et al*. Emergence of OXA-48-producing *Klebsiella*
392 *pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J Antimicrob Chemother*
393 2013; **68**: 317-21.
- 394 38. Turton JF, Doumith M, Hopkins KL *et al*. Clonal expansion of *Escherichia coli* ST38 carrying a
395 chromosomally integrated OXA-48 carbapenemase gene. *J Med Microbiol* 2016; **65**: 538-46.

- 396 39. Tarrant F, MacGowan AP, Walsh TR. Occurrence and current frequency of CTX-M-type beta-
397 lactamases from a regional hospital in the South West of England. *J Antimicrob Chemother* 2007; **59**:
398 815-6.
- 399 40. Wickramasinghe NH, Xu L, Eustace A *et al.* High community faecal carriage rates of CTX-M
400 ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. *J Antimicrob*
401 *Chemother* 2012; **67**: 1108-13.

402



405 **Figure 1.** Minimum spanning tree of the MLST profiles of 225 CTX-R *E. coli* isolates. The shaded areas represent single locus variants (SLVs).
 406 Members of the most prevalent STs (>4 representatives) are labelled and their number of representatives indicated. The diameter of the circle
 407 represents the number of isolates of that particular ST and the coloured segments indicate which CTX-R mechanisms were identified. Thick solid
 408 lines represent SLVs, thin solid lines represent double-locus variants and dashed connecting lines indicate multilocus variants. This figure appears
 409 in colour in the online version of JAC and in black and white in the printed version of JAC.

410

Isolates (M/F)	CTX-R mechanisms identified by PCR							CMY	DHA	SHV	None
	CTX-M G1	CTX-M G1 + DHA	CTX-M G1 + SHV	CTX-M G1 + CMY	CTX-M G9	CTX-M G8					
F (n=507)	334	3	1	2	123	1	6	11	6	20	
M (n=119)	77	1	2	1	26	0	4	2	2	4	
Total	626	4	3	3	149	1	10	13	8	24	

411

412 **Table 1.** Beta-lactamase genes detected by multiplex PCRs on 626 CTX-R isolates.

<i>bla</i> _{CTX-M} variant							<i>bla</i> _{CMY} variant				<i>bla</i> _{DHA} variant	<i>bla</i> _{SHV} variant
CTX-M-1	CTX-M-3	CTX-M-8	CTX-M-14	CTX-M-15	CTX-M-27	CTX-M-55	CMY-2	CMY-4	CMY-42	CMY-60	DHA-1	SHV-12
9	3	1	10	118	44	4	7	1	2	3	8	6

413

414 **Table 2.** ESBL/pAmpC variants identified in the 225 isolates subjected to WGS.

415 Note: 3 isolates harboured both *bla*_{CMY-60} and *bla*_{CTX-M-15}, and one isolate harboured *bla*_{DHA-1}
 416 and *bla*_{CTX-M-15}.

417

No. of isolates	<i>ampC</i> promoter/attenuator mutations	Pribnow box
8	-42C>T, -25G>A, -1C>T, +57C>T	TTGACA - 17nt - TATCGT
2	-28G>A, ins -12 T -13, +22G>T	TTGTCA - 17nt - TACAAT
2	-11C>T, ins -12 T -13, +33G>A, +36G>A	TTGTCA - 17nt - TATAAT
1	-32T>A, +34C>A, +57C>T	TTGACA - 16nt - TACAAT

418

419 **Table 3.** Mutations found within promoter/attenuator region of the 13 presumed AmpC-
 420 hyperproducing isolates subjected to WGS relative to *E. coli* MG1655 (Genbank Accession
 421 Number NC_000913.3).

422

423

424

ST131 Clades (no.)	CTX-R Alleles				
	CTX-M-1	CTX-M-14	CTX-M-15	CTX-M-27	CMY-60
A	11	1	4	5	
B	1	1			
C	C1-M27	23		23	
	C1-nM27	5	3	2	
	C2	54			
Unclassified	7		4 ^a	3	1 ^a
Total	101	2	4	62	33

425

426 **Table 4.** ST131 clades and CTX-R GOI alleles harboured by 101 isolates subject to WGS.

427 ^a One isolate belonging to an ST131 unclassified clade harboured both *bla*_{CTX-M-15} and *bla*_{CMY-}
428 60.