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

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

Abstract

Objectives: Third-generation cephalosporin-resistant *Escherichia coli* from community-acquired urinary tract infections (UTI) have been increasingly reported worldwide. In this study we sought to determine and characterise the mechanisms of cefotaxime-resistance (CTX-R) employed by urinary *E. coli* obtained from primary care over a 12-month period, in Bristol and surrounding counties in the South West of England.

Methods: Cephalexin resistant (Ceph-R) *E. coli* isolates were identified directly from general practice (GP) referred urine samples using disc susceptibility testing as per standard diagnostic procedures. CTX-R was determined by subsequent plating onto MIC breakpoint plates. β-Lactamase genes were detected by PCR. Whole Genome Sequencing (WGS) was performed on 225 urinary isolates and analyses were performed using the Centre for Genomic Epidemiology platform. Patient information provided by the referring GPs was reviewed.

Results: During the study period, Ceph-R *E. coli* (n=900) were obtained directly from urines from 146 GPs. Seventy-percent (626/900) of isolates were CTX-R. WGS of 225 non-duplicate isolates identified that the most common mechanism of CTX-R was *bla*$_{\text{CTX-M}}$ carriage (185/225; 82.2%), predominantly *bla*$_{\text{CTX-M-15}}$ (114/185; 61.6%), followed by carriage of plasmid mediated AmpCs (pAmpCs) (17/225; 7.6%), ESBL *bla*$_{\text{SHV}}$ variants (6/225; 2.7%), AmpC hyperproduction (13/225; 5.8%), or a combination of both *bla*$_{\text{CTX-M}}$ and pAmpC carriage (4/225; 1.8%). Forty-four sequence types (STs) were identified with ST131 representing 101/225 (45.0%) of sequenced isolates, within which the *bla*$_{\text{CTX-M-15}}$-positive clade C2 was dominant (54/101; 53.5%). Ciprofloxacin-resistance (CIP-R) was observed in 128/225 (56.9%) of sequenced CTX-R isolates – predominantly associated with fluoroquinolone-resistant clones ST131 and ST1193.

Conclusions: Most Ceph-R urinary *E. coli*s were CTX-R, predominantly caused by *bla*$_{\text{CTX-M}}$ carriage. There was a clear correlation between CTX-R and CIP-R, largely attributable to the

dominance of the high-risk pandemic clones, ST131 and ST1193 in this study. This localised epidemiological data provides greater resolution than regional data and can be valuable for informing treatment choices in the primary care setting.

Introduction

Escherichia coli that are resistant to β-lactam antibiotics, particularly to cephalosporins, represent a major global public health concern. Third-generation cephalosporins (3GCs), such as cefotaxime, are used across the world to treat infections caused by *E. coli* (e.g. urinary tract [UTIs], bloodstream and intra-abdominal infections) and subsequently the emergence of resistance is particularly worrisome.[1] Resistance to 3GCs in *E. coli* can be caused by multiple mechanisms including chromosomally encoded AmpC β-lactamase hyperproduction, and may involve increased efflux, and reduced outer membrane permeability, but is predominantly attributed to the spread of plasmid-mediated AmpC (pAmpC, e.g. bla_{CMY}) or extended-spectrum β-lactamases (ESBLs, e.g. $bla_{CTX\text{-}M}$).[2] *E. coli* which harbour ESBLs are often co-resistant to multiple antibiotic classes and subsequently the treatment options for such infections may be limited.[3]

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

E. coli sequence types (STs) belonging to phylogroups B2 (STs 73, 95 and 131) and D (ST69) have been reported to be major causes of both UTIs and bloodstream infections (BSIs) in the UK.[11] Since its initial description in 2008, numerous studies have shown that

the multidrug-resistant pandemic clone, ST131, is a major cause of UTI globally.[12-14] The ST131 clonal group can be broken down by population genetics into three clades based on their association with particular *fimH* types: A/*fimH*41, B/*fimH*22, and C/*fimH*30.[15] Clade C can be further broken down into four subclades; C1 – not usually associated with ESBL carriage but typically fluoroquinolone resistant (FQ-R), C1-M27 and C1-nM27 – both associated with $bla_{CTX\text{-}M\text{-}27}$ carriage and FQ-R, and C2 (also known as H30Rx) – associated with $bla_{CTX\text{-}M\text{-}15}$ carriage and FQ-R.[15, 16] Studies have suggested that the global dominance of ESBL-positive ST131 is, in part, due to its increased virulence potential over its non-ST131 ESBL-positive counterparts.[17, 18]

This study sought to use whole genome sequencing (WGS) to characterise the population structure and determine the mechanisms of CTX-R employed by urinary *E. coli* isolates referred from general practice in Bristol and surrounding counties in the South-West of England serving a population of approximately 1.2 million people.

Materials and Methods

Bacterial isolates, identification and susceptibility testing

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

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

Antibiotic susceptibilities were performed by disc testing or, in the case of colistin, by broth microdilution and interpreted according to EUCAST guidelines.[19] Ceph-R isolates were subcultured onto agar plates containing 2 mg/L cefotaxime (CTX) and isolates that were

124 positive for growth were deemed CTX-resistant (CTX-R), and taken forward for further
125 testing.

126 **Screening for β-lactamase genes**

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

131 **WGS and analyses**

132 WGS was performed by MicrobesNG (https://microbesng.uk/) on a HiSeq 2500 instrument
133 (Illumina, San Diego, CA, USA) using 2x250 bp paired end reads. Reads were trimmed
134 using Trimmomatic,[21] assembled into contigs using SPAdes[22] 3.13.0
135 (http://cab.spbu.ru/software/spades/) and contigs were annotated using Prokka.[23]
136 Resistance genes, plasmid replicon types, sequence types and *fim* types were assigned
137 using the ResFinder,[24] PlasmidFinder,[25] MLST[26] 2.0 and FimTyper on the Center for
138 Genomic Epidemiology (http://www.genomicepidemiology.org/) platform.

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

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

144 Plasmid pUB_DHA-1 was sequenced to closure and submitted to GenBank with accession
145 number MK048477. Reads were mapped using Geneious Prime 2019.1.3
146 (https://www.geneious.com).

147 **Plasmid Transformation**

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

Analysis of patient demographic information

Limited, non-identifiable patient information was obtained from the request forms sent with submissions from referring GP practices.

Results and Discussion

Patient demographics and antimicrobial susceptibilities

Nine hundred Ceph-R urinary *E. coli* isolates, obtained from primary care in Bristol and surrounding areas, were collected during the period of the study. Most isolates were obtained from female patients (721/900; 80.1%) and the average patient age was 63.2 years. Seventy-percent (626/900) were CTX-R and within these, most were again from females (507/626; 81.0%) with an average patient age of 63.6 years.

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

Table 1 indicates the number of CTX-R isolates carrying each β-lactamase genes of interest (GOIs; *bla*$_{CTX-Ms}$, *bla*$_{CMY}$, *bla*$_{DHA}$ or *bla*$_{SHV}$). *bla*$_{CTX-Ms}$ were by far the prevalent gene group, found in 571/626 (91.2%) of isolates. Within these *bla*$_{CTX-M-G1}$ were most common (421/626) followed by *bla*$_{CTX-M-G9}$ (149/626) and *bla*$_{CTX-M-G8}$ (one isolate). pAmpCs *bla*$_{CMY}$ and *bla*$_{DHA}$ were found in 13 (3 alongside *bla*$_{CTX-M-G1}$) and 17 (4 alongside *bla*$_{CTX-M-G1}$) isolates respectively. *bla*$_{SHV}$ was found in 11 (3 alongside *bla*$_{CTX-M-G1}$) isolates and the remaining 24 isolates harboured none of the GOIs as detected by PCR.

Whole Genome Sequencing (WGS) analyses

GOI variants and STs

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

CTX-R GOIs were identified in all but thirteen isolates (212/225; 94.2%). Eighty-four percent (189/225) of isolates harboured one of seven *bla*$_{\text{CTX-M}}$ gene variants (Table 2). Carriage of *bla*$_{\text{CTX-M-15}}$ was the most common CTX-R mechanism identified (118/189) followed by *bla*$_{\text{CTX-M-27}}$ (44/189) and *bla*$_{\text{CTX-M-14}}$ (10/189). Amongst the non-CTX-M GOIs, four *bla*$_{\text{CMY}}$ variants were identified; *bla*$_{\text{CMY-2}}$ (n=7), *bla*$_{\text{CMY-4}}$ (n=1), *bla*$_{\text{CMY-42}}$ (n=2) and *bla*$_{\text{CMY-60}}$ (n=3; all three being co-carried alongside *bla*$_{\text{CTX-M-15}}$), as well as *bla*$_{\text{DHA-1}}$ (n=8; one alongside *bla*$_{\text{CTX-M-15}}$) and *bla*$_{\text{SHV-12}}$ (n=6). The narrow spectrum β-lactamases *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM-1}}$ and inhibitor-resistant variant *bla*$_{\text{TEM-33}}$ were found in 53, 82, and one isolate respectively.

AmpC-hyperproducing isolates

All thirteen (5.8%) sequenced isolates, where no CTX-R GOI could be identified, were presumed to be chromosomal AmpC β-lactamase hyperproducers because they carry mutations within the *ampC* promoter/attenuator region previously seen in confirmed AmpC hyperproducers (Table 3).[28, 29] These represented nine different STs, each having one representative, with the exceptions of the STs 75 and 200 of which there were three representatives each (Table S2). This indicates a lack of dominant clones in AmpC hyperproducers identified in this study. If we go on to assume that the 24 isolates negative for GOIs by PCR are AmpC-hyperproducers, as was found with the thirteen representative sequenced isolates, then 3.8% of the isolates in this study could be classed as AmpC-

198 hyperproducers. Additionally, one *bla*$_{CTX-M-15}$-positive isolate was found to also harbour *ampC*
199 promoter changes associated with hyperproduction.

200 **Characterisation of ST131 Isolates**

201 **ESBLs and clades**

202 One hundred and one ST131 isolates harboured the following CTX-R mechanisms/alleles;
203 *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*$_{CTX-M-27}$
204 (n=33). The isolates were broken down into their respective clades (Table 4); ST131-C2 was
205 dominant (54/101; 53.5%) followed by C1-M27 (M27) (23/101; 22.8%), A (11/101; 10.9%),
206 C1-nM27 (5/101; 5.0%), one clade B, and seven isolates were unclassified. Eighty-eight
207 percent (89/101) of isolates, and notably all clade C2 isolates, were CIP-R as is a typical
208 characteristic of this lineage. Two non-ST131 members of the ST131 complex, both of which
209 were *bla*$_{CTX-M-15}$-positive ST8313 isolates (a *fumC* single locus variant (SLV) of ST131), also
210 harboured the same chromosomal FQ-R associated mutations in *gyrA*, *parC* and *parE* as
211 are associated with ST131/C2 – suggesting this ST may be ST131/C2 derived. Previous
212 studies have highlighted the dominance of ST131 and particularly the clade C2/H30Rx on a
213 worldwide scale.[13, 14] Since its initial description in 2008 in isolates from 3 continents,[12, 13]
214 ST131 has been reported across all inhabited continents.[15] The recently described C1
215 subclade, C1-M27, as characterised by the presence of a 11,894 bp prophage-like genomic
216 island M27PP1, was initially described in Japan in 2016 and has been reported in countries
217 in at least three continents: Europe, Asia and North America, so far.[16] The presence of C1-
218 M27 isolates in this study indicates the expansion of this particular ST131 sublineage into
219 the UK, similarly to that which has been reported from countries in mainland Europe.[30]

220 **Virotypes**

221 ST131 has been reported in previous studies to be a highly virulent clone, exhibiting lethality
222 in mouse sepsis models.[18, 31] Virotypes of all 101 ST131 isolates were determined as
223 previously described.[18, 32] Virotype C was most common, represented by 38/101 (37.6%) of

ST131 isolates and predominantly associated with *bla*$_{CTX-M-27}$ (28/38 isolates) across clades A (n=3), C1-M27 (n=22), C1-nM27 (n=4), and one isolate belonged to an unclassified clade. All *bla*$_{CTX-M-27}$-positive isolates belonged to virotype C, with the exception of one isolate for which a virotype could not be assigned. The association between virotype C and *bla*$_{CTX-M-27}$ carriage is in agreement with a recent study conducted in France.[33] Twenty-five isolates belonged to virotype A, all of which except one harboured *bla*$_{CTX-M-15}$. The remaining 38 isolates belonged to either virotype B (n=1), D (n=1), G (n=8), or were unknown virotypes (n=28).

Genetic context of ESBLs/pAmpC genes

Despite the limitations of short read sequencing, by examining the contigs on which GOIs were located, we were able to determine the chromosomal or plasmid environments of ESBLs/pAmpC genes in 85/225 isolates. Forty CTX-M genes, of variants *bla*$_{CTX-M-14}$ (n=3) and *bla*$_{CTX-M-15}$ (n=37), were found to be located on the chromosome. These were found in 11 STs with STs 131 (n=11) and 73 (n=10) being the most represented. Whilst the CTX-M genetic environments were diverse in ST131, in ST73 9/10 isolates harboured the gene in the same genomic location, suggesting a high degree of clonality within this ST. The remaining GOIs were found to be located within a relatively diverse range of plasmids and across multiple STs. Interestingly all six DHA-1-harbouring isolates were found to harbour a similar IncI1 plasmid which was sequenced to closure during this study, pUB_DHA-1. Read mapping analyses showed that all six isolates exhibited 95-100% coverage and 98-100% identity against pUB_DHA-1.

Other important resistance genes found by WGS

Interestingly one ST69 CMY-2-producing isolate was also found to harbour *mcr-1*. Susceptibility testing, performed by broth microdilution, revealed that the MIC of colistin against this isolate is 8 mg/L and so it is colistin resistant. Attempts at transformation of *E. coli* DH5 alpha using plasmid DNA extracted from this isolate were successful indicating that

mcr-1 is plasmid encoded. Analysis of the genetic environment of *mcr-1* found that it is encoded on an IncI2 plasmid of approximately 62 kb and it lacks the upstream IS*Apl1* element that was described in the initial discovery of *mcr-1* in China.[34] The plasmid itself does not encode any additional resistance genes and when subjected to NCBI BLAST analysis exhibited ~96% similarity to *mcr-1* encoding plasmids found in both China (pHNGDF93; Genbank Accession Number MF978388) and Taiwan (p5CRE51-MCR-1; Genbank Accession Number CP021176) from animal and human origins, respectively. Since initial reports of its discovery in 2015,[34] *mcr-1* has been reported worldwide in clinical *E. coli* isolates although remains relatively rare.

Another isolate, a $bla_{\text{CTX-M-14}}$-positive ST38, also encoded the $bla_{\text{OXA-48-like}}$ carbapenemase gene $bla_{\text{OXA-244}}$.[35] Transformation attempts using plasmid DNA from this isolate were unsuccessful and so it was concluded that $bla_{\text{OXA-244}}$ is likely to be chromosomally encoded. Disc susceptibility testing showed that this isolate is resistant to ertapenem but susceptible to both imipenem and meropenem. The presence of the chromosomally-encoded OXA-48-like carbapenemase $bla_{\text{OXA-244,}}$ confirms the observations of a previous study, where OXA-48-like genes were shown to have become embedded in the ST38 chromosome.[36] ST38 is the most frequent ST associated with OXA-48-like enzymes in the UK.[36]

Conclusions

Resistance to 3GCs and FQs in *E. coli* is of increasing concern due to the importance of these classes of drugs for the treatment of serious infections. As observed in this study and in line with previous reports globally,[2] the dissemination of successful clones is a major cause of CTX-R in urinary isolates from primary care in South West England.

The correlation between CIP-R and CTX-R highlighted here can be largely attributed to the dominance of successful clones/clades, namely ST131 and ST1193; the majority of both harbour chromosomal FQ-R mutations. Through WGS of a subset of isolates we have

shown that ST131 clade C2 is dominant and that the recently described ST131 subclade, C1-M27, is also prevalent despite not previously being described in the UK.

This study is the first analysis of CTX-R urinary *E. coli* derived from the community, and performed in a relatively localised area in South West England and can be useful for informing patient treatment as well as providing essential data for comparison purposes to other areas, both within and outwith the UK.

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Transparency declaration

None to declare.

Supplementary data

Tables S1 and S2 are available as supplementary data.

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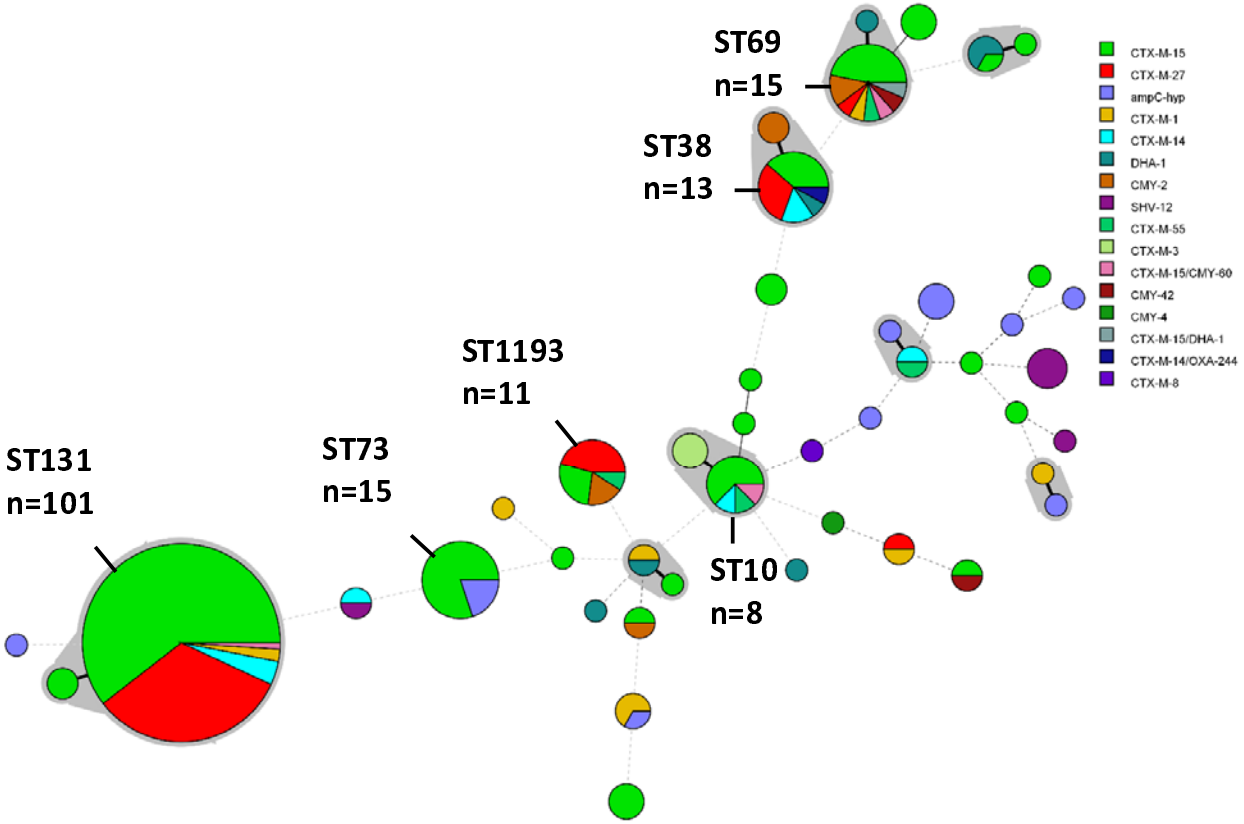

Figure 1. Minimum spanning tree of the MLST profiles of 225 CTX-R *E. coli* isolates. The shaded areas represent single locus variants (SLVs). Members of the most prevalent STs (>4 representatives) are labelled and their number of representatives indicated. The diameter of the circle represents the number of isolates of that particular ST and the coloured segments indicate which CTX-R mechanisms were identified. Thick solid lines represent SLVs, thin solid lines represent double-locus variants and dashed connecting lines indicate multilocus variants.

	Isolates (M/F)	CTX-R mechanisms identified by PCR									
		CTX-M G1	CTX-M G1 + DHA	CTX-M G1 + SHV	CTX-M G1 + CMY	CTX-M G9	CTX-M G8	CMY	DHA	SHV	None
	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**	**411**	**4**	**3**	**3**	**149**	**1**	**10**	**13**	**8**	**24**

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

bla$_{CTX-M}$ variant							*bla*$_{CMY}$ variant				*bla*$_{DHA}$ variant	*bla*$_{SHV}$ variant	None detected
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	NA
9	**3**	**1**	**10**	**118**	**44**	**4**	**7**	**1**	**2**	**3**	**8**	**6**	**13**

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

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

No. of isolates	*ampC* 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

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

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

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

[1] One isolate belonging to an ST131 unclassified clade harboured both *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CMY-60}}$.

424 **Supplementary Data**

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Primer	Sequence (5'-3')	Product size (bp)	Reference
CTX-M-G1F	AAAAATCACTGCGCCAGTTC	415	
CTX-M-G1R	AGCTTATTCATCGCCACGTT		
CTX-M-G2F	CGACGCTACCCCTGCTATT	552	
CTX-M-G2R	CCAGCGTCAGATTTTTCAGG		
CTX-M-G8F	TCGCGTTAAGCGGATGATGC	666	20
CTX-M-G9F	CAAAGAGAGTGCAACGGATG	205	
CTX-M-G9R	ATTGGAAAGCGTTCATCACC		
CTX-M-G25F	GCACGATGACATTCGGG	327	
CTX-M-G8/25R	AACCCACGATGTGGGTAGC		
CMY-F	CGATCCGGTCACGAAATACT	556	
CMY-R	CCAGCCTAATCCCTGGTACA		
DHA-F	GTGAAATCCGCCTCAAAAGA	341	
DHA-R	ACAATCGCCACCTGTTTTTC		
OXA-1-F	TTATCTACAGCAGCGCCAGT	451	This study.
OXA-1-R	AAGCTACTTTCGAGCCATGC		
SHV-F	CTTTCCCATGATGAGCACCT	127	
SHV-R	GCGAGTAGTCCACCAGATCC		
TEM-F	CCGAAGAACGTTTTCCAATG	249	
TEM-R	GTCCTCCGATCGTTGTCAGAA		
MCR-1_F	TGTTCTTGTGGCGAGTGTTG	468	
MCR-1_R	ACAGGCAGTAAAATCAGCGC		This study.
OXA-48-like_F	TCGATTTGGGCGTGGTTAAG	505	
OXA-48-like_R	AGCCCTAAACCATCCGATGT		

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427 **Table S1.** Primers used in this study.

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STs **(no. if >1)**	Beta-lactamase GOIs/mechanism identified **(no.)**
131 **(101)**	CTX-M-15 **(62)**,CTX-M-27 **(33)**,CTX-M-14 **(4)**,CTX-M-1 **(2),** CMY-60[1]
69 **(15)**	CTX-M-15 **(9)**,CTX-M-1,CTX-M-27,CTX-M-55,DHA-1[2],CMY-2 **(2)**,CMY-42,CMY-60[3]
73 **(15)**	CTX-M-15 **(12)**,AmpC-hyp **(3)**
38 **(13)**	CTX-M-15 **(5)**,CTX-M-27 **(4)**,CTX-M-14 **(3)**,DHA-1, OXA-244[4]
1193 **(11)**	CTX-M-27 **(5)**,CTX-M-15 **(3)**,CMY-2 **(2)**,CTX-M-55
10 **(8)**	CTX-M-15 **(6)**,CTX-M-55,CMY-60[5], CTX-M-14
359 **(4)**	SHV-12 **(4)**
141 **(3)**	CTX-M-1 **(2)**,AmpC-hyp
200 **(3)**	AmpC-hyp **(3)**
209 **(3)**	CTX-M-3 **(3)**
349 **(3)**	DHA-1 **(2)**,CTX-M-15
394 **(3)**	CTX-M-15 **(3)**
636 **(3)**	CTX-M-15 **(3)**
12 **(2)**	CTX-M-14, SHV-12
58 **(2)**	CTX-M-14,CTX-M-55
80 **(2)**	CTX-M-15,CMY-2
127 **(2)**	CTX-M-1,DHA-1
405 **(2)**	CTX-M-15 **(2)**
648 **(2)**	CTX-M-15,CMY-42
963 **(2)**	CMY-2 **(2)**
1722 **(2)**	CTX-M-1,CTX-M-27
8313 **(2)**	CTX-M-15 **(2)**
23	CTX-M-1
54	AmpC-hyp
75	AmpC-hyp
88	AmpC-hyp
95	CTX-M-15
155	AmpC-hyp
162	SHV-12
224	CTX-M-15
428	AmpC-hyp
443	CTX-M-15
448	AmpC-hyp
457	CMY-4
491	DHA-1
540	CTX-M-8
1431	CTX-M-15
2015	CTX-M-1
2914	CTX-M-15
3036	DHA-1
4981	CTX-M-15
7401	DHA-1
8312	CTX-M-15
8467	CTX-M-15

Table S2. The STs and CTX-R mechanisms identified in 225 isolates subjected to WGS.

[1] – CMY-60 harboured alongside CTX-M-15.

[2] – DHA-1 harboured alongside CTX-M-15.

[3] – CMY-60 harboured alongside CTX-M-15.

[4] – OXA-244 harboured alongside CTX-M-14.

[5] – CMY-60 harboured alongside CTX-M-15.