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Molecular epidemiology of cefotaxime-resistant Escherichia coli from dairy farms in South West England identifies a dominant plasmid encoding CTX-M-32

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Molecular epidemiology of *Escherichia coli* producing CTX-M and plasmid AmpC-type β-lactamases from dairy farms identifies a dominant plasmid encoding CTX-M-32 but no evidence for transmission to humans in the same geographical region

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Running heading: Cefotaxime-resistant *E. coli* from dairy farms

Abstract

Third-generation cephalosporin resistance (3GC-R) in *Escherichia coli* is a rising problem in human and farmed animal populations. We conducted whole genome sequencing analysis of 138 representative 3GC-R isolates previously collected from dairy farms in South West England and confirmed by PCR to carry acquired 3GC-R genes. This analysis identified $bla_{CTX\text{-}M}$ (131 isolates: encoding CTX-M-1, -14, -15, -32 and the novel variant, CTX-M-214), $bla_{CMY\text{-}2}$ (6 isolates) and $bla_{DHA\text{-}1}$ (one isolate). A highly conserved plasmid was identified in 73 isolates, representing 27 *E. coli* sequence types. This novel ~220 kb IncHI2 plasmid carrying $bla_{CTX\text{-}M\text{-}32}$ was sequenced to closure and designated pMOO-32. It was found experimentally to be stable in cattle and human transconjugant *E. coli* even in the absence of selective pressure and was found by multiplex PCR to be present on 26 study farms representing a remarkable range of transmission over 1500 square kilometres. However, the plasmid was not found amongst human urinary *E. coli* we have recently characterised from people living in the same geographical location, collected in parallel with farm sampling. There were close relatives of two $bla_{CTX\text{-}M}$ plasmids circulating amongst eight human and two cattle isolates, and a closely related $bla_{CMY\text{-}2}$ plasmid found in one cattle and one human isolate. However, phylogenetic evidence of recent sharing of 3GC-R strains between farms and humans in the same region was not found.

Importance

Third-generation cephalosporins (3GCs) are critically important antibacterials and 3GC-resistance (3GC-R) threatens human health, particularly in the context of opportunistic pathogens such as *Escherichia coli*. There is some evidence for zoonotic transmission of 3GC-R *E. coli* through food, but little work has been done

examining possible transmission (e.g. via interaction of people with the local near-farm environment). We characterised acquired 3GC-R *E. coli* found on dairy farms in a geographically restricted region of the United Kingdom and compared these with *E. coli* from people living in the same region, collected in parallel. Whilst there is strong evidence for recent farm-to-farm transmission of 3GC-R strains and plasmids – including one epidemic plasmid that has a remarkable capacity to transmit – there was no evidence that 3GC-R found on study farms had a significant impact on circulating 3GC-R *E. coli* strains or plasmids in the local human population.

Introduction

Third-generation cephalosporin-resistant (3GC-R) *Escherichia coli* have been increasingly reported in both animal and human populations and are considered pathogens of major concern for humans (1,2). 3GCs such as cefotaxime and ceftazidime have been listed by the World Health Organisation (WHO) as "highest-priority critically important antimicrobials" (HP-CIAs) because of their importance for human health (3). Resistance to 3GCs in *E. coli* can be caused by several mechanisms but is primarily attributed to the acquisition of Extended Spectrum β-Lactamases (ESBLs) or plasmid-mediated AmpC β-lactamases (pAmpCs) (4). Plasmids encoding ESBLs and pAmpCs frequently harbour additional resistance genes and so can present a significant therapeutic challenge (5). In recent years, the promotion and implementation of the 'One Health' approach in antimicrobial resistance by the WHO has emphasised the importance of surveillance in both animal and human populations and has highlighted gaps in this knowledge (6). In humans it has been well established in numerous global studies that certain *E. coli* lineages (e.g. *bla*$_{\text{CTX-M}}$-encoding ST131) play a major role in the dissemination of ESBL genes, however such a depth of information does not exist for isolates from animal populations (2). Human-associated pandemic lineages have been reported in animal populations albeit to a much lesser extent than in human populations (7).

In humans, *bla*$_{\text{CTX-M}}$ variants are the globally dominant ESBL type with some variants exhibiting geographical associations (e.g. *bla*$_{\text{CTX-M-15}}$ in Europe and North America and *bla*$_{\text{CTX-M-14}}$ in Asia) (2). Transmission of ESBLs occurs largely through horizontal gene transfer, with conjugative IncF plasmids being reported as the dominant vehicles for *bla*$_{\text{CTX-M}}$ genes (8,9). Previous studies using typing methodologies including WGS have suggested transmission of both strains and ESBL plasmids

77 across animal and human populations (10,11). Epidemic plasmids have been
78 reported across different host populations and in multiple countries (12). For
79 example, one epidemic plasmid type – pCT, encoding *bla*$_{\text{CTX-M-14}}$ – was identified in
80 cattle and human *E. coli* isolates in England and found to exist in human isolates
81 from several countries across 3 continents (12).

82 Antimicrobial use in food animals may provide selective pressure for resistance
83 genes/plasmids which could theoretically be spread to humans (13). However,
84 recent reports suggest that such transmission is limited, at least in the UK (14). In
85 dairy farming, antibiotics are used both therapeutically in the treatment of common
86 infections such as mastitis, and preventatively. For example, in so-called dry cow
87 therapy, an antibacterial preparation inserted into a cow's udder between lactations
88 to prevent mastitis (15). A survey of dairy farms in England and Wales in 2012
89 revealed that the fourth-generation cephalosporin (4GC) cefquinome (another HP-
90 CIA) was the most used dry cow therapy treatment (16). By 2017, however, only
91 5.3% of total dry cow therapy active ingredients were HP-CIAs. Indeed, there has
92 been a significant decline in the use of HP-CIAs on dairy farms in the UK in recent
93 years (17).

94 Recently, we reported a survey of 53 dairy farms located in South West England
95 where we investigated the prevalence of 3GC-R *E. coli* (18). From 1226 such
96 isolates, PCR analysis confirmed that 648/1226 (52.7%) carried *bla*$_{\text{CTX-M}}$ and
97 13/1226 (1.1%) carried a pAmpC gene. The remaining 566/1226 (46.2%) isolates did
98 not carry any putative acquired 3GC-R gene, and a subsequent analysis confirmed
99 that hyper-production of the chromosomally encoded AmpC β-lactamase was the
100 mechanism of 3GC-R in these isolates (19).

101 The study reported here aimed to characterise, using WGS, representative *bla*$_{CTX-M}$
102 and pAmpC-positive *E. coli* isolates collected on dairy farms during our earlier
103 surveillance study (18). Furthermore, our aim was to compare these isolates at strain
104 and plasmid-encoded 3GC-R gene level with *bla*$_{CTX-M}$ and pAmpC positive urinary *E.*
105 *coli* isolates (20) collected from humans living in the same 50 x 50 km region that
106 was the location of the majority of dairy farms under study.

107

108 **Results and Discussion**

109 **WGS analysis of *E. coli* carrying acquired 3GC-R genes from dairy farms**

110 One hundred and thirty-eight representative isolates, PCR-positive for *bla*$_{CTX-M}$ or
111 pAmpC genes (18) and chosen to give coverage of all 42 farms positive for acquired
112 3GC-R genes, were subjected to WGS (**Table 1**). *bla*$_{CTX-M-32}$, encoding a group 1
113 enzyme first described in a human clinical isolate in 2004 (21), was the most
114 common 3GC-R gene identified and was found in 79/138 sequenced isolates
115 encompassing 27 *E. coli* sequence types (STs) from 25 farms Other 3GC-R genes
116 identified were: *bla*$_{CTX-M-14}$ (18 isolates, 6 STs from 9 farms), *bla*$_{CTX-M-1}$ (16 isolates, 8
117 STs from 6 farms), *bla*$_{CTX-M-15}$ (16 isolates, 5 STs from 10 farms), *bla*$_{CMY-2}$ (6 isolates,
118 3 STs from 3 farms), *bla*$_{CTX-M-214}$ (3 isolates, 2 STs from 3 farms) plus one isolate
119 harbouring *bla*$_{DHA-1}$ and one isolate having both *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-14}$. CTX-M-214
120 (GenBank Accession No. MH121688) is a novel CTX-M-9 variant, first identified in
121 this study, which differs from CTX-M-9 by a single amino acid, Ala112Thr. In all three
122 isolates encoding *bla*$_{CTX-M-214}$, the gene was identified on a contig which also
123 encoded an IncI-ST26 plasmid replicon as well as *aadA2*, *sul1*, and *dfrA16*.

124

Identification and characterisation of pMOO-32

Following observations of the high prevalence of *bla*$_{CTX-M-32}$, a search for common plasmid replicon types was conducted which revealed an IncHI2-ST2 replicon in almost all the sequenced *bla*$_{CTX-M-32}$-positive isolates. Transconjugations were attempted into *E. coli* DH5α using *bla*$_{CTX-M-32}$-positive farm isolate DK as donor (**Table 2**). One successful transconjugant was sent for WGS employing both long and short read methodologies to sequence the plasmid to closure. pMOO-32 is a 226,022-bp conjugative plasmid belonging to the ST2-IncHI2 incompatibility group, harbouring *repHI2* and *repHI2A* replication genes. It contains 245 putative ORFs and has a GC content of 45.5% (**Figure 1**). pMOO-32 encodes the following antimicrobial resistance genes: *bla*$_{CTX-M-32}$, *strA*, *strB*, *aph(6)-Ic*, *aph(3')-IIa* and *tetB* as well as genes encoding resistance to the heavy metal compound, tellurite (*terABCDEFWXYZ*) and a HipAB type II toxin-antitoxin system along with a second partial system (*higB* toxin gene). *bla*$_{CTX-M-32}$ is encoded downstream of an IS*ECp1* element within which there is an IS*Kpn26* insertion encoded in the opposite orientation (**Figure 2**). This same genetic environment was also observed in 4 *bla*$_{CTX-M-32}$-positive but IncHI2 plasmid-negative ST10 isolates collected from 2 farms. There were 2 additional IncHI2 plasmid-negative ST765 isolates, both from the same farm, that carried *bla*$_{CTX-M-32}$ where the immediate genetic environment differed by a truncation in IS*Ecp1*.

Transconjugation attempts using the pMOO-32-positive farm isolate DK as donor into a 3GC-susceptible (3GC-S) cattle ST88 *E. coli* isolated from one of the study farms (18) as well as into a 3GC-S human urinary ST1193 *E. coli* isolate (20) were both successful (**Table 2**). ST1193 was selected as a recently described fluoroquinolone-resistant global clone, often implicated as a cause of human

150 infections (22), whilst ST88 was selected as a particularly prevalent ST among cattle
151 isolates (23). Antimicrobial disc testing showed that the pMOO-32-carrying donor
152 was, as expected from the genotype, resistant to ampicillin, cefotaxime, cefepime,
153 aztreonam, streptomycin, neomycin, and tetracycline. The cattle ST88 and human
154 ST1193 transconjugants were, additional to their starting wild-type resistance profile,
155 resistant to cefotaxime, cefepime and aztreonam. These results (**Table 2**) are
156 indicative of the functionality of the *bla*$_{\text{CTX-M-32}}$ gene harboured by pMOO-32.

157

158 **Epidemiology of pMOO-32-like plasmids on farms**

159 The complete nucleotide sequence of pMOO-32 was submitted to GenBank under
160 accession number MK169211. Using this sequence as a reference, sequencing
161 reads from all 73 isolates positive for *bla*$_{\text{CTX-M-32}}$ and the IncHI2 replicon were
162 mapped; this indicated that the plasmids exhibited 94-100% identity to the reference
163 sequence. Any differences could be attributed to a loss or gain of mobile genetic
164 elements, but no major rearrangements were observed to the plasmid backbone or
165 changes to resistance gene content. These 73 isolates carrying pMOO-32-like
166 plasmids comprised 27 *E. coli* STs. On 10 farms, pMOO-32-like plasmids were found
167 in isolates of more than one ST. The most frequently identified STs were ST69 and
168 ST10, found in 18 isolates from 7 farms, and 6 isolates from 4 farms, respectively.
169 Therefore, we conclude that pMOO-32-like plasmids are dominant in this study area,
170 largely a result of horizontal rather than clonal transmission.

171 We subsequently designed a multiplex PCR, based on the pMOO-32 sequence, and
172 used to it screen all group 1 *bla*$_{\text{CTX-M}}$-PCR-positive isolates from our study farms (18)
173 not previously subjected to WGS for the presence of pMOO-32-like plasmids. It was

found that 26/53 (49.1%) farms within our study area tested positive for the presence of a pMOO-32-like plasmid using this test. The origins and geographical reach of pMOO-32 remain to be established, but all positive farms were located in a 40 x 40 km sub-region of the wider study area, where 26/33 farms were positive, suggesting that the plasmid remained geographically confined at the time of sample collection.

We hypothesised that the observed dominance of pMOO-32-like plasmids could be a consequence of the HipAB-type II toxin-antitoxin system found on the plasmid, leading to persistence even in the absence of antibacterial selective pressure. Growth curve assays indicated a 12-40% fitness cost (reduction in OD_{600}) of pMOO-32 carriage in *E. coli* DH5α at the end of the exponential growth phase in M9 minimal medium (data not shown). However, despite this cost in growth terms, pMOO-32 was stably maintained over 10 days of passaging in the absence of antibiotic pressure in the farm isolates, their respective DH5α transconjugants, and the human and cattle *E. coli* isolate transconjugants tested.

No evidence for recent sharing of 3GC-R *E. coli* and limited evidence for recent sharing of 3GC-R *E. coli* plasmids between humans and dairy farms

The ability of pMOO-32 to readily transfer into human urinary *E. coli* ST1193 (**Table 2**) and be maintained in the absence of antimicrobial pressure indicates the zoonotic potential of this plasmid. We next aimed to see if there was evidence of recent sharing of 3GC-R isolates or plasmids, including pMOO-32, between dairy farms and humans living in the same geographical region as the farms. To do this, we compared WGS data from farm isolates described above with data from human urinary *E. coli* collected in parallel within the same 50 x 50 km geographical region

198 (20). Since 10 dairy farms under study here lie outside the 50 x 50 km region (18),
199 isolates from these farms were excluded.

200 Core genome phylogenetic analysis of 324 sequenced (112 dairy farm and 212
201 human) *E. coli* isolates carrying acquired 3GC-R genes collected within the target
202 region (**Figure 3**) revealed only four STs including examples of both farm and human
203 isolates. In no case was the single nucleotide polymorphism (SNP) difference
204 between any pair of human and farm isolate core genomes suggestive of recent
205 transmission. ST10 was the closest (≥205 SNPs different between human and cattle
206 isolates; **Figure 3** insert); the others were ST540 (929 SNPs different), ST58 (≥1388
207 SNPs different) and ST69 (≥831 SNPs different). In contrast, there was clear
208 evidence of recent farm-to-farm transmission of isolates from multiple STs (e.g.
209 ST10 and ST69 where, in both cases, there was a 3 SNP minimum distance
210 between pairs of isolates representing two farms) (**Figure 3**). AmpC hyper-producing
211 *E. coli* isolates from these same farms showed a similar pattern: no evidence of
212 strain sharing between dairy farms and humans but strong evidence for recent farm-
213 to-farm transmission (19). We conclude therefore that if humans encounter 3GC-R
214 isolates from farms, such isolates do not readily go on to colonise them and/or cause
215 UTI.

216 We next considered transmission of plasmid-mediated 3GC-R between farm and
217 human *E. coli* isolates (i.e. those that that have caused UTI). We found that 37/107
218 $bla_{CTX\text{-}M}$-positive *E. coli* isolates from farm samples within our 50 x 50 km study
219 region carried $bla_{CTX\text{-}M}$ variants also seen amongst 189 $bla_{CTX\text{-}M}$-positive urinary *E.*
220 *coli* cultured from people living in the same region during the same time period (20).
221 None of the human isolates carried $bla_{CTX\text{-}M\text{-}32}$ or any plasmid related to pMOO-32.
222 By filtering sequenced isolates by their $bla_{CTX\text{-}M}$ variants and plasmid replicon types,

223 plasmids that shared high degrees of sequence identity in farm and human isolates
224 were identified. One IncI1-ST3 plasmid, found in an ST345 farm isolate, harboured
225 *bla*$_{CTX-M-1}$. Mapping of sequencing reads showed that this plasmid exhibited 100%
226 coverage and 97.5% sequence identity to an unpublished ~106 kb IncI1-ST3
227 plasmid (pTC_N40607; GenBank Accession No. CP007651) found in *E. coli*
228 obtained from meat/cattle isolates in the USA. Six human urinary *E. coli* isolates
229 representing STs 23, 127, 131, 141 and 2015 harboured *bla*$_{CTX-M-1}$ on a plasmid that
230 exhibited 99.4-100% coverage and 96.4-98.7% identity when sequencing reads were
231 mapped to pTC_N40607.

232 Another plasmid type - again obtained from a single farm isolate, in this case of
233 ST58 - exhibited 100% coverage and 98.5% identity by read mapping to a published
234 IncK plasmid, pCT (GenBank Accession No. NC_014477). pCT is ~94 kb and
235 harbours *bla*$_{CTX-M-14}$. pCT-like plasmids have been reported in both human and
236 veterinary *E. coli* isolates across three continents (12, 23). Amongst human urinary
237 *E. coli* isolates found in this study, two also carried pCT-like plasmids. Both isolates
238 were the pandemic clone ST131, and their pCT-like plasmids exhibited 96.4 or
239 97.2% identity and 100% coverage to pCT.

240 In terms of plasmids encoding pAmpC genes, we identified a *bla*$_{CMY-2}$ encoding
241 plasmid, p96 (GenBank Accession no. CP023370). This is a ~96 kb IncI1 plasmid
242 derived from an *E. coli* that caused urinary tract infections in dogs in Scotland (24).
243 Mapping analyses showed that one out of seven *bla*$_{CMY-2}$-carrying human urinary
244 isolates (ST 80) showed 100% coverage and 99.9% identity to p96. An almost
245 identical plasmid was found in one farm isolate (ST1480) that showed 99.6%
246 coverage and 98.9% identity to p96. As with the two *bla*$_{CTX-M}$ plasmids, this *bla*$_{CMY-2}$
247 encoding plasmid appears to be globally widespread. Blast analysis found *bla*$_{CMY-2}$

248 plasmids with >95% coverage and >98% identity to p96 in *E. coli* isolates from
249 humans in Taiwan (25) and chicken meat in the USA (GenBank Accession no.
250 CP048295).
251
252 **Concluding remarks**

253 Overall, this analysis finds no evidence of recent, direct sharing of *E. coli* between
254 farms and the local human population that have resulted in UTI. However, three farm
255 *E. coli* isolates carried a $bla_{CTX\text{-}M}$ or $bla_{CMY\text{-}2}$ plasmid almost identical to one of three
256 plasmids found in urinary *E. coli* in the local human population. However, these three
257 plasmids are known to be widely disseminated in humans and animals across
258 several continents. Furthermore, no human/farm plasmid pair shared 100% identity.
259 So, whilst there is some general evidence of shared circulating plasmids, as reported
260 in a number of recent studies (14,26,27), the overall level of overlap between UK
261 dairy farm and human 3GC-R *E. coli* identified in this study was very small and not
262 suggestive of any novel or recent zoonotic transmission events. In contrast, both
263 recent and sustained farm-to-farm transmission of 3GC-R *E. coli*, and particularly of
264 a newly identified epidemic plasmid pMOO-32 across many different *E. coli* STs and
265 dairy farms, was clearly seen. Identifying the vectors for this transmission will inform
266 interventions that might facilitate a more rapid reduction in 3GC-R *E. coli* on dairy
267 farms.
268
269 **Experimental**

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

Details of farm sample collection and microbiological analysis has recently been reported (18). In brief, samples of faecally contaminated sites were collected using sterile overshoes on 53 dairy farms located in South West England between January 2017 and December 2018. Samples were plated onto TBX agar (Sigma-Aldrich, Poole, UK) containing 16 $mg.L^{-1}$ cephalexin. Up to 5 *E. coli* colonies per cefalexin plate were re-plated onto TBX agar containing 2 $mg.L^{-1}$ cefotaxime to confirm resistance. Presence of acquired 3GC-R genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{CMY-2}$ and *bla*$_{DHA-1}$) was confirmed by PCR. Disc susceptibility testing was performed and interpreted according to EUCAST guidelines (28).

Transconjugations

Transconjugations were performed using rifampicin-resistant (Rif-R) *E. coli* DH5α with both human and cattle *E. coli* isolates as the recipients (**Table 3**). Briefly, 1 mL each of overnight broth cultures of donor and recipient cells were mixed in a 3:1 ratio before centrifugation and resuspension in 50 µL of PBS. Five microlitre aliquots were spotted onto LB agar (Oxoid, Basingstoke, UK) plates and incubated at 37°C for 6 h. Growth was collected and resuspended in 100 µL of PBS before being plated on MacConkey agar (Oxoid) plates containing either 32 $mg.L^{-1}$ rifampicin (for Rif-R *E. coli* DH5α) or 0.5 $mg.L^{-1}$ ciprofloxacin (for strains HC4 and HG), and 2 $mg.L^{-1}$ cefotaxime. Transconjugant colonies were screened by PCR.

WGS and analyses and pMOO-32 PCR

Representative isolates were selected for WGS based on resistance phenotype, β-lactamase gene carriage and farm of isolation, as defined previously (18). WGS was

295 performed by MicrobesNG (https://microbesng.uk/) on a HiSeq 2500 instrument
296 (Illumina, San Diego, CA, USA) using 2x250 bp paired end reads. Reads were
297 trimmed using Trimmomatic (29) and assembled into contigs using SPAdes 3.13.0
298 (30) (http://cab.spbu.ru/software/spades/). Resistance genes, plasmid replicon types
299 and sequence types (according to the Achtman scheme [31]) were assigned using
300 the ResFinder (32), PlasmidFinder (33), and MLST 2.0 on the Center for Genomic
301 Epidemiology (http://www.genomicepidemiology.org/) platform. Enhanced genome
302 sequencing (combining Illumina and MinION reads) was performed by MicrobesNG
303 on one transconjugant and reads were assembled using Unicycler (34). Contigs
304 were annotated using Prokka 1.2 (35).

305 Reads and assembled contigs were aligned to reference sequences using the
306 progressive Mauve alignment software (36), CLC Genomics Workbench 12 (Qiagen,
307 Manchester, UK), or BWA (37) and SAMtools (38), with variant positions being called
308 using BCFtools (39). pMOO-32 was visualised using the CGView server (40)
309 (http://stothard.afns.ualberta.ca/cgview_server/).

310 A multiplex PCR, targeting five size-distinguishable regions of pMOO-32, was
311 designed to indicate the presence of pMOO-32-like plasmids (**Table 4**).

312

313 **Plasmid stability assay**

314 Three representative pMOO-32 PCR-positive isolates, obtained from different farms,
315 and their transconjugant counterparts were subjected to 10 days of serial passaging
316 on non-selective LB agar. After 10 days, colonies were screened for the presence of
317 pMOO-32 by PCR.

318

Fitness cost assay

Fitness cost was assessed by a growth curve assay using M9 minimal medium (Sigma-Aldrich). Rif-R *E. coli* DH5α and the pMOO-32 transconjugant strain were grown with shaking at 37°C and OD_{600} measurements were taken at hourly intervals. Assays were performed on three biological replicates.

Phylogenetic analysis

Sequence alignment and phylogenetic analysis was carried out using the Bioconda environment (41) on the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) (42). The reference sequence was *E. coli* ST131 isolate EC958 complete genome (accession: HG941718). Sequences were first aligned to a closed reference sequence and analysed for SNP differences, whilst omitting insertion and deletion elements, using the Snippy alignment program. Alignment was then focused on regions of the genome found across all isolates, the core genome, using the Snippy-core program, thus eliminating the complicating factors of insertions and deletions (https://github.com/tseemann/snippy). Aligned sequences were then used to construct a maximum likelihood phylogenetic tree using RAxML, utilising the GTRCAT model of rate heterogeneity and the software's autoMR and rapid bootstrap to find the best-scoring maximum likelihood tree and including tree branch lengths, defined as the number of base substitutions per site compared (43,44). Finally, phylogenetic trees were illustrated using the web-based Microreact program (45).

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Transparency declaration

The authors declare no conflict of interests. Farming businesses who permitted access to collect the isolates studied here were not involved in the design of this study or in data analysis and were not involved in drafting the manuscript for publication.

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Conceived the Study: K.K.R., M.B.A.

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Initial Drafting of Manuscript: J.F., M.B.A.

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Abundance of acquired 3GC-R mechanisms identified by WGS and number of STs/farms involved														
Total isolates/ farms	*bla*$_{CTX-M-32}$	STs/ farms	*bla*$_{CTX-M-1}$	STs/ farms	*bla*$_{CTX-M-14}$	STs/ farms	*bla*$_{CTX-M-15}$	STs/ farms	*bla*$_{CTX-M-214}$	STs/ farms	*bla*$_{DHA-1}$	STs/ farms	*bla*$_{CMY-2}$	STs/ farms
138/42	79	27/25	16[a]	8/6	18[a]	6/9	16	5/10	3	2/3	1	1/1	6	3/3

Table 1. Characteristics of 3GC-R 138 isolates subjected WGS.

[a]One isolate harboured both *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-14}$.

	Zone Diameters (mm)													
Antimicrobial Agent	*E. coli* DK	S/I/R	*E. coli* DH5α	S/I/R	*E. coli* DH5α TR	S/I/R	*E. coli* HG	S/I/R	*E. coli* HG TR	S/I/R	*E. coli* HC4	S/I/R	*E. coli* HC4 TR	S/I/R
AMP	<6	R	30	S	<6	R	<6	R	<6	R	20	S	<6	R
CTX	10	R	45	S	18	I	35	S	10	R	34	S	13	R
CAZ	20	I	45	S	30	S	34	S	20	I	32	S	23	S
FEP	19	R	45	S	28	S	28	S	18	R	35	S	24	I
ETP	30	S	45	S	44	S	34	S	33	S	36	S	36	S
ATM	15	R	45	S	24	I	34	S	15	R	35	S	19	R
STR[a]	<6	R	25	S	10	R	<6	R	<6	R	13	R	<6	R
TOB	17	S	28	S	26	S	<6	R	<6	R	18	S	19	S
NEO[a]	8	R	20	S	12	R	<6	R	<6	R	14	I	8	R
TET[a]	<6	R	36	S	<6	R	<6	R	<6	R	30	S	<6	R

Table 2. Disc susceptibility testing of *E. coli* DK and pMOO-32 transconjugants of various *E. coli* recipient strains.

AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; ATM, aztreonam; STR, streptomycin; TOB, tobramycin; NEO, neomycin; TET, tetracycline; TR, pMOO-32 transconjugant.

[a]Streptomycin and neomycin sensitivities were determined using tobramycin EUCAST interpretation guidelines, and tetracycline according to guidelines for *Yersinia enterocolitica*.

Isolate	Source/Host	ST	Resistance Genes	Use
DK	Cattle	155	*strA*, *strB*, *aph(6)-Ic*, *aph(3')-IIa*, *tet(B)*, $bla_{CTX\text{-}M\text{-}32}$	Donor
HC4	Human	1193	*aadA5*, *dfrA17*, *mdf(A)*, *sul1*	Recipient
HG	Cattle	88	*aph(6)-Id*, *ant(2'')-Ia*, *aph(3')-Ia*, *aadA24*, *aph(3'')-Ib*, *sul1*, *sul2*, *tet(A)*, *tet(B)*, $bla_{TEM\text{-}1}$, $bla_{OXA\text{-}1}$, *catA1*, *floR*	Recipient

Table 3. Characteristics of *E. coli* strains used in transconjugation experiments.

Primer	Sequence (5'-3')	Product Size (bp)	Target
aph(3')-IIa_F	TGGCTACCCGTGATATTGCT	642	*aph(3')-IIa*/*aph(6)-Ic* junction
aph(6)-Ic_R	CTGGCGGACGGGAAGTATC		
HI2A_F	AGCCTTTCTCACGGTAGCAT	526	HI2 *repA*
HI2A_R	TTCAATTGTCGGTGAGCGTC		
TraI_F	CGGGAAAAGCTGCACTCAAT	396	*traI*
TraI_R	AAGACTTTGTGAGCTTGGCG		
TetB_F	TTCAGCGCAATTGATAGGCC	285	*tetB*
TetB_R	ATCCCACCACCAGCCAATAA		
CTX-M-32_F	TTAGGAAGTGTGCCGCTGTA	180	$bla_{\text{CTX-M-32}}$
CTX-M-32_R	CACGGCCATCACTTTACTGG		

Table 4. Primers used for the pMOO-32 multiplex PCR.

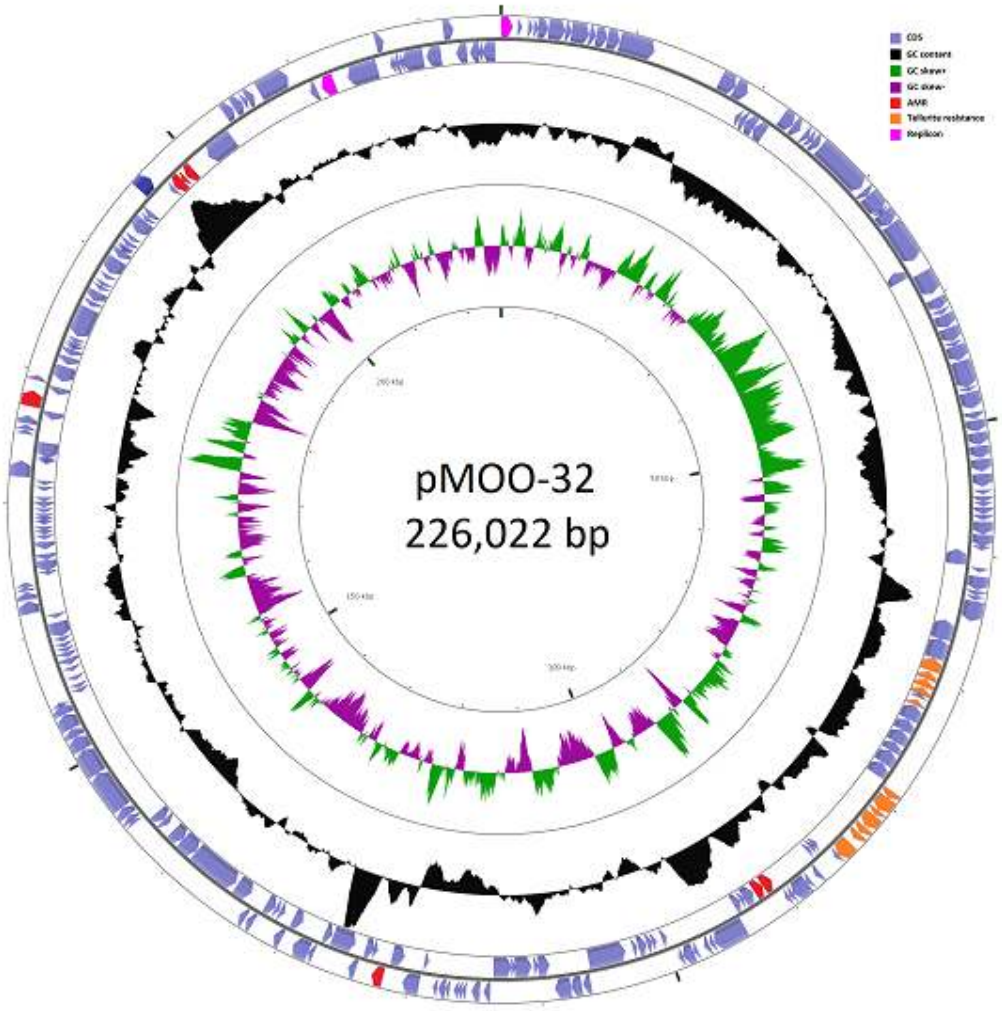

Figure 1. Plasmid pMOO-32 created using CGView (40).

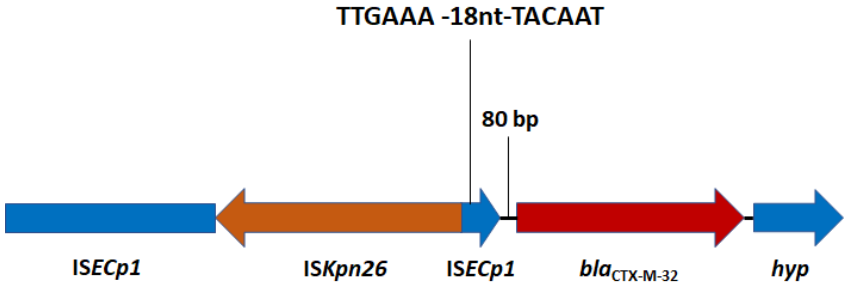

Figure 2. The genetic environment of *bla*$_{\text{CTX-M-32}}$ in pMOO-32 and other IncHI2 positive, *bla*$_{\text{CTX-M-32}}$-harbouring isolates.

Figure 3. Phylogenetic analysis of *E. coli* from dairy farms and human UTI collected in parallel in a 50 x 50 km region.

Human isolates are noted in orange; cattle isolates are noted in blue. The reference ST131 isolate is noted in black. Certain key STs are highlighted, particularly STs with representatives from human and cattle isolates: ST21 (ST540), ST69, ST58 and ST10. The insert shows a more detailed analysis of ST10 isolates which represents the closest relationship between a human and a cattle isolate: 205 SNPs different across the core genome.