Chromosomal integration of *bla*$_{CTX-M}$ genes in diverse *Escherichia coli* isolates recovered from river water in Japan

Ryota Gomi[a*], Masaki Yamamoto[b], Michio Tanaka[b], Yasufumi Matsumura[b]

[a]Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, 615-8540, Kyoto, Japan

[b]Department of Clinical Laboratory Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin-kawahara-cho, Sakyo-ku, 606-8507, Kyoto, Japan

Running title: Chromosomal location of *bla*$_{CTX-M}$ in environmental *E. coli*

[*]Corresponding author

E-mail address: gomi.ryota.34v@kyoto-u.jp; Tel: +81-75-383-3354; Fax: +81-75-383-3358

16 **ABSTRACT**

17 Occurrence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (ESBLEC)
18 in environmental waters is of great concern. However, unlike clinical ESBLEC, their genetic
19 characteristics, in particular the genetic contexts of ESBL genes, are not well understood. In
20 this study, we sequenced and analyzed the genomes of CTX-M-producing *E. coli* isolates
21 recovered from river water to fully characterize the genetic contexts of *bla*$_{CTX-M}$ genes. Among
22 the 14 isolates with completed genomes, *bla*$_{CTX-M}$ genes were detected on the chromosome in
23 nine isolates. All but one chromosomal *bla*$_{CTX-M}$ genes were associated with IS*Ecp1* and were
24 carried on different transposition units (TPU) ranging in size from 2,855 bp to 11,093 bp; the
25 exception, *bla*$_{CTX-M-2}$, was associated with IS*CR1*. In many cases, the TPU were directly
26 inserted into chromosomes. However, in one case, the TPU was part of a 36-kbp
27 multiresistance region bracketed by IS*26*, and the whole multiresistance region was inserted
28 into the chromosome. We also found a case where a chromosomally-located TPU and an
29 adjacent region had transposed into another chromosomal location, leading to duplication of
30 *bla*$_{CTX-M}$. The remaining five isolates carried *bla*$_{CTX-M}$ genes on epidemic IncI1 plasmids of
31 different STs (ST3, ST16, ST113, and ST167) (n = 4) or on an IncB/O/K/Z plasmid (n = 1).
32 This study revealed that environmental *E. coli* carry *bla*$_{CTX-M}$ genes in diverse genetic contexts.
33 Apparent high prevalence of chromosomal *bla*$_{CTX-M}$ potentially indicates that some

34 environmental *E. coli* can stably maintain *bla*$_{CTX-M}$ genes in the absence of antibiotic selection

35 pressure, though further studies are needed to confirm this.

36

37

38 **INTRODUCTION**

39 Occurrence of antibiotic-resistant bacteria (ARB) in surface waters is a public health concern.

40 Among ARB, extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* (ESBLEC)

41 represent a major threat to public health, because ESBLEC can be important causes of

42 community-onset infections for which treatment options are limited (1, 2). ESBL enzymes are

43 divided into several families (e.g., TEM, SHV, and CTX-M), among which CTX-M enzymes

44 have been recognized as the most common ESBL type (3). ESBL genes are often found on

45 plasmids and associated with mobile genetic elements such as transposons or insertion

46 sequences, enabling their movement within or between DNA molecules (3, 4). However, some

47 previous studies also detected ESBL genes on chromosomes and suggested that integration of

48 ESBL genes into chromosomes could contribute to the maintenance of ESBL genes in the

49 absence of antibiotic selection pressure (5-7). Although previous studies have reported the

50 occurrence of ESBLEC in environmental waters, limited information exists regarding the

51 locations (chromosome or plasmid) and genetic contexts of ESBL genes in environmental

52 ESBLEC (8-11).

53 In our previous work, we employed Illumina short-read sequencing technologies and
54 characterized 155 *E. coli* isolates obtained from the Yamato River in Japan (12). Among the
55 sequenced isolates, 18 carried *bla*$_{CTX-M}$ genes. However, the locations and genetic contexts of
56 the *bla*$_{CTX-M}$ genes could not be determined due to (i) the presence of repeat sequences in the
57 genomes and (ii) the presence of multiple dead ends in the short-read assembly graphs. Here,
58 the genomes of those 18 *E. coli* isolates with *bla*$_{CTX-M}$ genes were sequenced again with
59 Illumina short-read sequencing technology and also with Oxford Nanopore Technologies
60 (ONT) long-read sequencing platforms, aiming to elucidate the locations and genetic contexts
61 of the *bla*$_{CTX-M}$ genes. The genetic contexts of chromosomal *bla*$_{CTX-M}$ genes in the genomes of
62 environmental *E. coli* retrieved from the NCBI Reference Sequence Database (RefSeq) were
63 also characterized.

64

65 **MATERIALS AND METHODS**

66 **Bacterial isolates.**

67 We previously collected 531 *E. coli* isolates from the Yamato River in Japan between 2011
68 and 2013, and sequenced a subset of isolates (n = 155) using Illumina short-read sequencing
69 technology (12). Among the 155 isolates, 18 found to carry *bla*$_{CTX-M}$ genes were subjected to
70 the following analysis.

71 **Antibiotic susceptibility testing.**

72 Antibiotic susceptibility was assessed by microdilution using the dry plate Eiken assay (Eiken,
73 Tokyo, Japan) as described previously (see **Table S1** for antimicrobial agents used) (12). The
74 results were interpreted using CLSI criteria (13). ESBL production was confirmed following
75 the CLSI guidelines (13).

76 **Genome sequencing and assembly.**

77 DNA was extracted from each isolate using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden,
78 Germany). The short-read sequencing library was prepared using the Illumina DNA Prep
79 (Illumina, San Diego, CA), and the library was sequenced on an Illumina NovaSeq 6000 system
80 for 500 cycles (250-bp paired-end reads). The long-read sequencing library was prepared using
81 the SQK-LSK109 kit (Oxford Nanopore Technologies, Oxford, UK), and the prepared library
82 was sequenced on the MinION with a FLO-MIN106 flow cell.

83 Illumina short reads were subsampled using seqtk (v1.3, https://github.com/lh3/seqtk), and the
84 subsampled reads were trimmed with fastp (v0.20.0) (14). ONT long reads were filtered with
85 Filtlong (v0.2.0, https://github.com/rrwick/Filtlong) to remove reads with low quality or short
86 length. Hybrid assembly of Illumina short reads and ONT long reads was performed by
87 employing a short-read-first approach, i.e., Unicycler (v0.4.8) with the --no_correct option (15).
88 Hybrid assembly was also performed using a long-read-first approach, i.e., long-read assembly
89 by Flye (v2.9) (16) followed by long-read polishing using Medaka (v1.4.4,
90 https://github.com/nanoporetech/medaka) and short-read polishing using Polypolish (v0.4.3)

91 (17). Assembly graphs of both hybrid assemblies were visualized using Bandage (v0.8.1) for
92 each isolate (18), and a better assembly was chosen as the final assembly.

93 **Genomic analysis.**

94 Genomes were annotated using the RAST server (19), ISfinder (20), ResFinder 4.1 (21), and
95 the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmid replicons were
96 detected using PlasmidFinder 2.1 (22). Relaxase types, mate-pair formation types, and
97 transferability of plasmids were determined using MOB-typer (23). IncI1 plasmids were
98 annotated using plasmids R64, ColIb-P9, and R621a as references (24).

99 **Characterization of environmental *E. coli* genomes with *bla*$_{CTX-M}$ genes in RefSeq.**

100 RefSeq *E. coli* genomes with an assembly level of "complete genome" (n = 1903) were
101 downloaded in January 2022. Metadata information, such as collection date and isolation
102 source, was extracted from the GenBank files using an in-house Python script, and genomes of
103 *E. coli* from environmental waters were retrieved (n = 53). Antibiotic resistance genes were
104 detected using ABRicate (https://github.com/tseemann/abricate), and genomes carrying
105 *bla*$_{CTX-M}$ on the chromosome were analyzed as described above.

106 **Data availability.**

107 The complete genomes and sequence read files were deposited in GenBank and the NCBI SRA
108 under BioProject PRJNA800231 (also see **Table S2** for the accession number of each replicon

109 for the completed genomes and **Table S3** for SRA accession numbers of the remaining

110 genomes).

111

112 **RESULTS AND DISCUSSION**

113 **Basic characteristics of *E. coli* isolates sequenced in this study.**

114 All 18 *bla*$_{CTX-M}$-carrying isolates characterized in the present study were non-susceptible to

115 various antimicrobial agents such as ampicillin, cefpodoxime, and cefotaxime (**Table S1**).

116 ESBL production was confirmed in all the isolates. The 18 isolates were highly diverse,

117 comprising 16 different STs (see **Table S2** and **Table S3**). The *bla*$_{CTX-M}$ genes detected were

118 also diverse, namely, *bla*$_{CTX-M-14}$ (n = 8), *bla*$_{CTX-M-2}$ (n = 2), *bla*$_{CTX-M-15}$ (n = 2), *bla*$_{CTX-M-24}$ (n

119 = 2), *bla*$_{CTX-M-55}$ (n = 2), *bla*$_{CTX-M-1}$ (n = 1), *bla*$_{CTX-M-3}$ (n = 1), and *bla*$_{CTX-M-8}$ (n = 1). One isolate

120 (KTa008) carried two copies of the *bla*$_{CTX-M-14}$ gene.

121 Of the 18 isolates, the genomes of 14 could be completed. These genomes carried 0 to 8

122 plasmids (see **Table S2** for details). The genomes of the remaining four isolates could not be

123 completed, probably due to the presence of long repeats in the genomes (see **Table S3** for

124 information on the draft assemblies of these genomes).

125 **Genetic contexts of chromosomal *bla*$_{CTX-M}$ genes.**

126 The location and genetic contexts of the *bla*$_{CTX-M}$ genes were determined for the 14 isolates

127 with completed genomes. Of these 14, nine carried *bla*$_{CTX-M}$ genes on chromosomes and five

128 carried *bla*$_{CTX-M}$ genes on plasmids.

129 In eight of the nine isolates which carried *bla*$_{CTX-M}$ genes on chromosomes, the *bla*$_{CTX-M}$ genes

130 were associated with IS*Ecp1* and carried on transposition units (TPU) of different sizes (2,855

131 bp to 11,093 bp) (**Figure 1**). These TPU were flanked by AT-rich 5-bp target site duplications

132 (TSD). None of the TPU were identical, and the insertion sites of the TPU were different in all

133 cases, indicating that different transposition events contributed to the chromosomal integration

134 of the TPU. Notably, the TPU in KFu023 contained multiple resistance genes, including *bla*$_{CTX-}$

135 $_{M-15}$, *aac(6')-Ib-cr*, *bla*$_{OXA-1}$, and *ΔcatB3*. This seems to highlight the ability of IS*Ecp1* to

136 capture sequences of different lengths in different transposition events and thus simultaneously

137 mobilize antibiotic resistance genes of different origins (4). In most cases, these TPU were

138 directly inserted into chromosomes. However, in KKa019, the TPU was part of a 35,625 bp

139 multiresistance region bracketed by two copies of IS*26* (annotated as IS*15DI* in the ISFinder

140 database), and the whole multiresistance region was flanked by 8-bp TSD (CGTTGCCG) in

141 the chromosome (**Figure S1**). KTa008 carried two copies of *bla*$_{CTX-M-14}$, which were separated

142 by ~1,169 kbp in the chromosome. Analysis of the genetic contexts of these *bla*$_{CTX-M-14}$ genes

143 indicated transposition of an already chromosomally-located TPU and an adjacent region into

144 another chromosomal location (**Figure S2**). This type of 'recurrent' transposition was
145 previously reported in clinical ESBLEC, though the TPU and insertion sites were different (25).
146 We searched for *E. coli* genomes in the public database which carry the same TPU in the same
147 chromosomal location as those identified in the present study. BLASTN searches were
148 performed against the nucleotide collection (nr/nt) database in January 2022 using the TPU and
149 surrounding sequences identified in the present study as query sequences. Three of the nine
150 TPU/insertion site combinations were found in the database, while the remaining six were not
151 found, potentially corresponding to novel integration events (**Table S4**).
152 In KOr019, *bla*$_{\text{CTX-M-2}}$ was associated with IS*CR1* and situated within a complex class 1
153 integron, which is congruent with previous studies (4, 26). This complex class 1 integron was
154 closely related to a recently reported transposon Tn*7337* (**Figure S3**) (27). KOr019 carried the
155 IRt (inverted repeat at *tni* end)-IS*6100*-IRt structure, but this structure was truncated by IS*26*
156 in Tn*7337*. The 17,747 bp structure from IRi (inverted repeat at *intI*1 end) to IRt was inserted
157 into the chromosome of KOr019, creating 5-bp TSD of AAATG. This insertion seems to have
158 been mediated by Tni proteins provided *in trans*, since the *tni* genes in the complex class 1
159 integron were absent (4).
160 **Genetic contexts of *bla*$_{\text{CTX-M}}$ genes on plasmids.**
161 The five completed plasmids with *bla*$_{\text{CTX-M}}$ genes belonged to IncI1 (n = 4) and IncB/O/K/Z (n
162 = 1) (**Figure 2a**). IncI1 plasmids were typed as four different STs, namely ST3, ST16, ST113,

163 and ST167, by IncI1 pMLST (22). TPU were inserted into plasmid backbones in the IncI1/ST3,
164 IncI1/ST16, and IncI1/ST167 plasmids. Interestingly, the IncI1/ST16 and IncI1/ST167
165 plasmids carried the same TPU (except for insertion of IS*1* in the IncI1/ST16 plasmid) inserted
166 into the same location. IncI1/ST167 is a single-locus variant of IncI1/ST16, which indicates
167 the TPU was inserted into the plasmid backbone before the ST diversification. In the
168 IncI1/ST113 plasmid, an IS26-ΔIS*10R*-*bla*$_{CTX-M-8}$-IS*26* structure was inserted into the plasmid.
169 BLASTN analysis identified IncI1 plasmids belonging to the same IncI1-STs and carrying the
170 same TPU/IS*26* structures inserted into the same locations from different countries (e.g.,
171 MH847453 from France for *bla*$_{CTX-M-1}$-carrrying IncI1/ST3, CP056650 from the UK for
172 *bla*$_{CTX-M-55}$-carrrying IncI1/ST16, KY964068 from Portugal for *bla*$_{CTX-M-8}$-carrrying
173 IncI1/ST113, CP021208 from China for *bla*$_{CTX-M-55}$-carrrying IncI1/ST167), which indicates
174 circulation of epidemic CTX-M IncI1 plasmids in geographically distant countries.
175 Comparison of IncI1 plasmids revealed the replication region, the leading region, and the
176 transfer regions in the IncI1 reference plasmid R64 were well conserved in all IncI1 plasmids
177 detected in this study, while many genes in the stability region were lacking (**Figure 2b**). In
178 the IncB/O/K/Z plasmid, *bla*$_{CTX-M-14}$ was linked to ΔTn*1721* and a class 1 integron. Similar
179 genetic contexts were previously reported (e.g., JF701188).

180 **Chromosomal location of *bla*$_{CTX-M}$ in the RefSeq assemblies.**

181 Among the 53 RefSeq environmental *E. coli* genomes, 20 carried *bla*$_{CTX-M}$. Sixteen carried
182 *bla*$_{CTX-M}$ on plasmids and four carried *bla*$_{CTX-M}$ on chromosomes (**Table S5**). The four genomes
183 with chromosomal *bla*$_{CTX-M}$ belonged to ST38 (n = 2), ST130 (n = 1) and ST648 (n = 1) and
184 originated from various countries (Japan, New Zealand, Norway, and Switzerland), which
185 indicates diverse *E. coli* isolates carrying chromosomal *bla*$_{CTX-M}$ are also present in
186 environmental waters in other countries. In all four genomes, *bla*$_{CTX-M}$ was associated with
187 TPU ranging from 2,971 bp to 13,552 bp. A TPU containing multiple resistance genes besides
188 *bla*$_{CTX-M}$ and a TPU inserted into a multiresistance region were detected, again highlighting the
189 importance of these mechanisms in the emergence of multidrug resistant *E. coli* in the
190 environment (see **Figure S4** for the analysis of the genetic contexts of *bla*$_{CTX-M}$ in these
191 genomes).
192 ST38, which was detected in two RefSeq genomes containing chromosomal *bla*$_{CTX-M}$, was also
193 detected in two isolates with chromosomal *bla*$_{CTX-M}$ sequenced in this study. Interestingly,
194 ST38 was also found to be prevalent in some previous studies reporting chromosomal *bla*$_{CTX-}$
195 $_{M}$ (6, 28, 29). The reason for the prevalence of ST38 among *E. coli* with chromosomal *bla*$_{CTX-}$
196 $_{M}$ is unknown. However, multiple integration events seem to have been involved in the
197 acquisition of chromosomal *bla*$_{CTX-M}$ in ST38 (see **Figure 1**, **Figure S1**, and **Figure S4**), which
198 potentially indicates ST38 has genetic mechanisms that can promote chromosomal integration
199 of *bla*$_{CTX-M}$.

200 **Conclusions.**

201 Here, we demonstrated that the genetic contexts of *bla*$_{CTX-M}$ genes in environmental *E. coli* are
202 highly diverse in terms of the associated mobile elements (various TPU, IS*CR1*, and IS*26*) and
203 integration sites. This study also revealed that the chromosomal locations of *bla*$_{CTX-M}$ genes
204 were unexpectedly frequent in environmental ESBLEC in Japan. Chromosomal integration of
205 *bla*$_{CTX-M}$ might allow *E. coli* to stably maintain *bla*$_{CTX-M}$ in the absence of antibiotic selection
206 pressure in environmental waters, though further studies are needed to confirm this.

207

208 **Acknowledgements**

209 We acknowledge the NGS core facility of the Genome Information Research Center at the
210 Research Institute for Microbial Diseases of Osaka University for the support in DNA
211 sequencing. Computations were partially performed on the NIG supercomputer at ROIS
212 National Institute of Genetics. This work was supported by the Environment Research and
213 Technology Development Fund (JPMEERF20205006) of the Ministry of the Environment,
214 Japan. This study was also supported in-part by the Program for the Development of Next-
215 generation Leading Scientists with Global Insight (L-INSIGHT), sponsored by the Ministry of
216 Education, Culture, Sports, Science and Technology (MEXT), Japan.

217

218

219 References

220 1. Pitout JD, Laupland KB. 2008. Extended-spectrum beta-lactamase-producing
221 Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8:159-66.
222 2. Pitout JD. 2010. Infections with extended-spectrum beta-lactamase-producing
223 enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs 70:313-
224 33.
225 3. Castanheira M, Simner PJ, Bradford PA. 2021. Extended-spectrum beta-lactamases: an
226 update on their characteristics, epidemiology and detection. JAC Antimicrob Resist
227 3:dlab092.
228 4. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile Genetic Elements
229 Associated with Antimicrobial Resistance. Clin Microbiol Rev 31.
230 5. Hirai I, Fukui N, Taguchi M, Yamauchi K, Nakamura T, Okano S, Yamamoto Y. 2013.
231 Detection of chromosomal *bla*$_{\text{CTX-M-15}}$ in *Escherichia coli* O25b-B2-ST131 isolates
232 from the Kinki region of Japan. Int J Antimicrob Agents 42:500-6.
233 6. Rodriguez I, Thomas K, Van Essen A, Schink AK, Day M, Chattaway M, Wu G,
234 Mevius D, Helmuth R, Guerra B, consortium S-E. 2014. Chromosomal location of
235 *bla*$_{\text{CTX-M}}$ genes in clinical isolates of *Escherichia coli* from Germany, The Netherlands
236 and the UK. Int J Antimicrob Agents 43:553-7.
237 7. Nair S, Chattaway M, Langridge GC, Gentle A, Day M, Ainsworth EV, Mohamed I,
238 Smith R, Jenkins C, Dallman TJ, Godbole G. 2021. ESBL-producing strains isolated
239 from imported cases of enteric fever in England and Wales reveal multiple
240 chromosomal integrations of *bla*$_{\text{CTX-M-15}}$ in XDR *Salmonella* Typhi. J Antimicrob
241 Chemother 76:1459-1466.
242 8. Jang J, Suh YS, Di DY, Unno T, Sadowsky MJ, Hur HG. 2013. Pathogenic *Escherichia*
243 *coli* strains producing extended-spectrum beta-lactamases in the Yeongsan River basin
244 of South Korea. Environ Sci Technol 47:1128-36.
245 9. Zurfluh K, Hachler H, Nuesch-Inderbinen M, Stephan R. 2013. Characteristics of
246 extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae
247 Isolates from rivers and lakes in Switzerland. Appl Environ Microbiol 79:3021-6.
248 10. Muller A, Stephan R, Nuesch-Inderbinen M. 2016. Distribution of virulence factors in
249 ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and
250 humans. Sci Total Environ 541:667-672.
251 11. Girlich D, Bonnin RA, Naas T. 2020. Occurrence and Diversity of CTX-M-Producing
252 *Escherichia coli* From the Seine River. Front Microbiol 11:603578.
253 12. Gomi R, Matsuda T, Matsumura Y, Yamamoto M, Tanaka M, Ichiyama S, Yoneda M.
254 2017. Whole-Genome Analysis of Antimicrobial-Resistant and Extraintestinal
255 Pathogenic *Escherichia coli* in River Water. Appl Environ Microbiol 83:e02703-16.

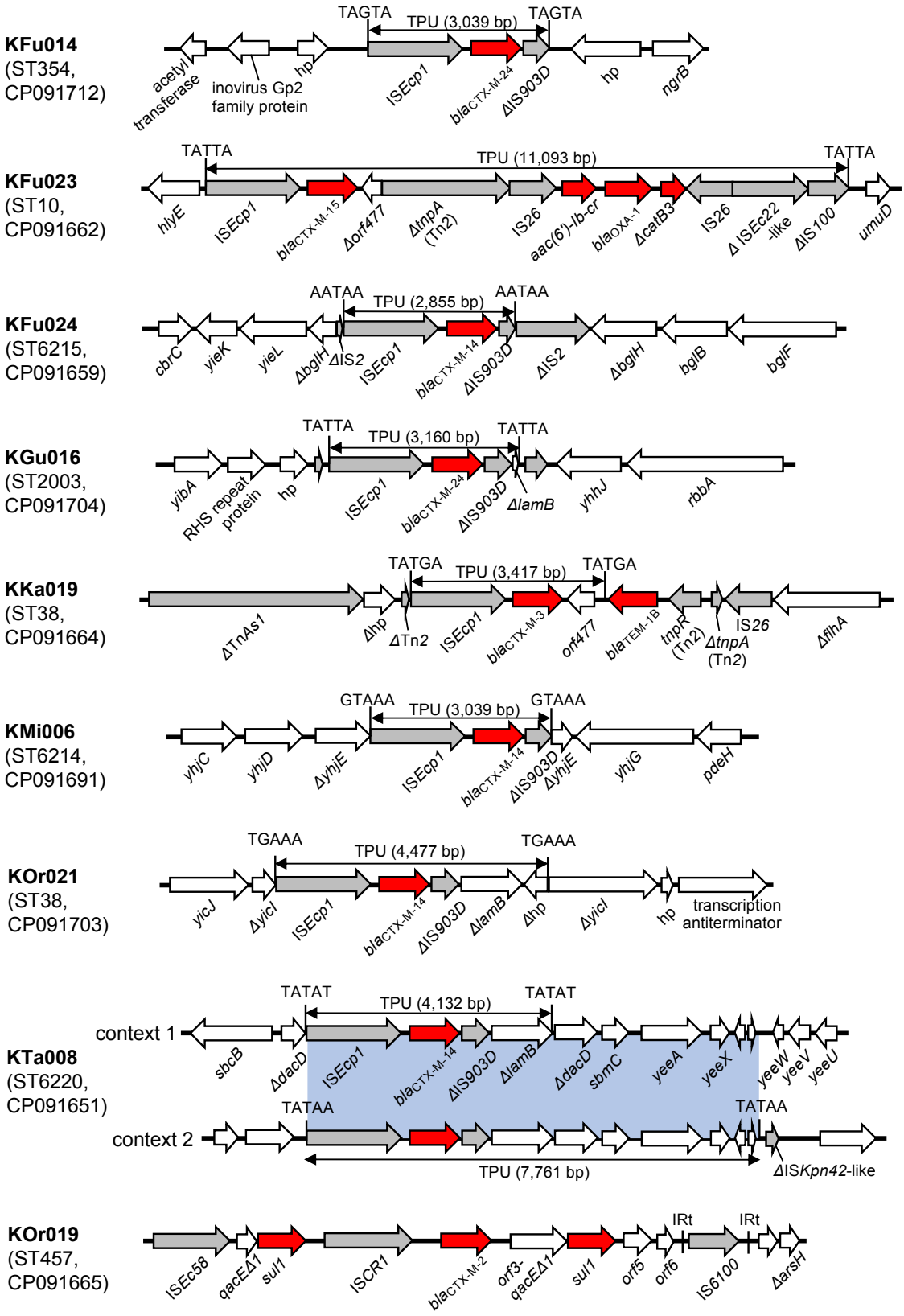
256 13. CLSI. 2020. *Performance Standards for Antimicrobial Susceptibility Testing—*
257 *Thirtieth Edition: M100*.
258 14. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ
259 preprocessor. Bioinformatics 34:i884-i890.
260 15. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome
261 assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595.
262 16. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads
263 using repeat graphs. Nat Biotechnol 37:540-546.
264 17. Wick RR, Holt KE. 2022. Polypolish: Short-read polishing of long-read bacterial
265 genome assemblies. PLoS Comput Biol 18:e1009802.
266 18. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of
267 de novo genome assemblies. Bioinformatics 31:3350-2.
268 19. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes
269 S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA,
270 McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R,
271 Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid
272 annotations using subsystems technology. BMC Genomics 9:75.
273 20. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the
274 reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32-6.
275 21. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A,
276 Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B,
277 Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar
278 S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykasenoja S, Olkkola S,
279 Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O,
280 Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J
281 Antimicrob Chemother 75:3491-3500.
282 22. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller
283 Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using
284 PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother
285 58:3895-903.
286 23. Robertson J, Nash JHE. 2018. MOB-suite: software tools for clustering, reconstruction
287 and typing of plasmids from draft assemblies. Microb Genom 4:e000206.
288 24. Carattoli A, Villa L, Fortini D, Garcia-Fernandez A. 2021. Contemporary IncI1
289 plasmids involved in the transmission and spread of antimicrobial resistance in
290 Enterobacteriaceae. Plasmid 118:102392.
291 25. Hamamoto K, Hirai I. 2019. Characterisation of chromosomally-located *bla*$_{\text{CTX-M}}$ and
292 its surrounding sequence in CTX-M-type extended-spectrum beta-lactamase-producing
293 *Escherichia coli* isolates. J Glob Antimicrob Resist 17:53-57.

294 26. Canton R, Gonzalez-Alba JM, Galan JC. 2012. CTX-M Enzymes: Origin and
295 Diffusion. Front Microbiol 3:110.
296 27. Coppola N, Cordeiro NF, Trenchi G, Esposito F, Fuga B, Fuentes-Castillo D, Lincopan
297 N, Iriarte A, Bado I, Vignoli R. 2021. Imported One-Day-Old Chicks as Trojan Horses
298 for Multidrug-Resistant Priority Pathogens Harboring *mcr-9, rmtG* and Extended-
299 Spectrum beta-Lactamase Genes. Appl Environ Microbiol doi:10.1128/AEM.01675-
300 21:AEM0167521.
301 28. Guiral E, Mendez-Arancibia E, Soto SM, Salvador P, Fabrega A, Gascon J, Vila J.
302 2011. CTX-M-15-producing enteroaggregative *Escherichia coli* as cause of travelers'
303 diarrhea. Emerg Infect Dis 17:1950-3.
304 29. Guenther S, Semmler T, Stubbe A, Stubbe M, Wieler LH, Schaufler K. 2017.
305 Chromosomally encoded ESBL genes in *Escherichia coli* of ST38 from Mongolian
306 wild birds. J Antimicrob Chemother 72:1310-1313.
307 30. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment
308 with gene gain, loss and rearrangement. PLoS One 5:e11147.

309

310

311 **FIGURES**



312

313 **Figure 1**. Genetic contexts of chromosomal *bla*$_{CTX-M}$ genes. Red arrows indicate antibiotic

314 resistance genes, gray arrows indicate mobile elements, and white arrows indicate other

315 genes. The blue shaded box indicates the regions with 100% nucleotide identity. The entire

316 resistance regions of KKa019 and KOr019 are shown in **Figure S1** and **Figure S3**,

317 respectively. 5-bp TSD are shown next to each TPU. hp: hypothetical protein.

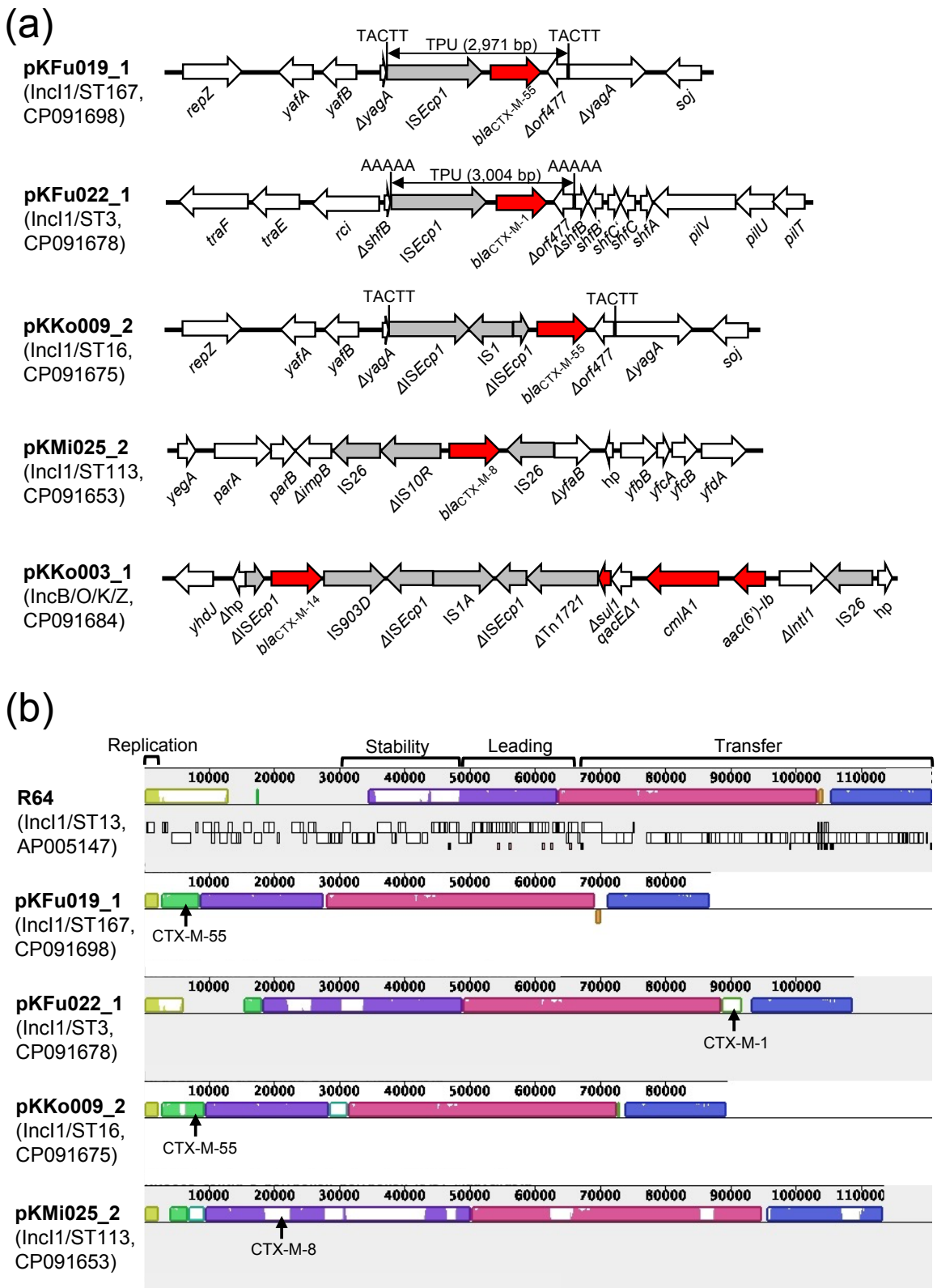


Figure 2. (a) Genetic contexts of *bla*$_{CTX-M}$ genes on plasmids. (b) Mauve comparison of IncI1 plasmids (30). IncI1 reference plasmid R64 is shown at the top. Four major regions in R64

321 (replication, stability, leading, and conjugative transfer) are indicated. Homologous segments

322 are shown as colored blocks. Locations of *bla*$_{\text{CTX-M}}$ genes are indicated with arrows.