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

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ABSTRACT

Occurrence of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* (ESBLEC) in environmental waters is of great concern. However, unlike clinical ESBLEC, their genetic characteristics, in particular the genetic contexts of ESBL genes, are not well understood. In this study, we sequenced and analyzed the genomes of CTX-M-producing *E. coli* isolates recovered from river water to fully characterize the genetic contexts of *bla*_{CTX-M} genes. Among the 14 isolates with completed genomes, *bla*_{CTX-M} genes were detected on the chromosome in nine isolates. All but one chromosomal *bla*_{CTX-M} genes were associated with *ISEcp1* and were carried on different transposition units (TPU) ranging in size from 2,855 bp to 11,093 bp; the exception, *bla*_{CTX-M-2}, was associated with *ISCR1*. In many cases, the TPU were directly inserted into chromosomes. However, in one case, the TPU was part of a 36-kbp multiresistance region bracketed by *IS26*, and the whole multiresistance region was inserted into the chromosome. We also found a case where a chromosomally-located TPU and an adjacent region had transposed into another chromosomal location, leading to duplication of *bla*_{CTX-M}. The remaining five isolates carried *bla*_{CTX-M} genes on epidemic IncI1 plasmids of different STs (ST3, ST16, ST113, and ST167) (n = 4) or on an IncB/O/K/Z plasmid (n = 1). This study revealed that environmental *E. coli* carry *bla*_{CTX-M} genes in diverse genetic contexts. Apparent high prevalence of chromosomal *bla*_{CTX-M} potentially indicates that some

environmental *E. coli* can stably maintain *bla*_{CTX-M} genes in the absence of antibiotic selection pressure, though further studies are needed to confirm this.

INTRODUCTION

Occurrence of antibiotic-resistant bacteria (ARB) in surface waters is a public health concern. Among ARB, extended-spectrum- β -lactamase (ESBL)-producing *Escherichia coli* (ESBLEC) represent a major threat to public health, because ESBLEC can be important causes of community-onset infections for which treatment options are limited (1, 2). ESBL enzymes are divided into several families (e.g., TEM, SHV, and CTX-M), among which CTX-M enzymes have been recognized as the most common ESBL type (3). ESBL genes are often found on plasmids and associated with mobile genetic elements such as transposons or insertion sequences, enabling their movement within or between DNA molecules (3, 4). However, some previous studies also detected ESBL genes on chromosomes and suggested that integration of ESBL genes into chromosomes could contribute to the maintenance of ESBL genes in the absence of antibiotic selection pressure (5-7). Although previous studies have reported the occurrence of ESBLEC in environmental waters, limited information exists regarding the locations (chromosome or plasmid) and genetic contexts of ESBL genes in environmental ESBLEC (8-11).

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

MATERIALS AND METHODS

Bacterial isolates.

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

Antibiotic susceptibility testing.

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

Genome sequencing and assembly.

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

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

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

Genomic analysis.

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

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

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

Data availability.

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

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

RESULTS AND DISCUSSION

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

All 18 *bla*_{CTX-M}-carrying isolates characterized in the present study were non-susceptible to various antimicrobial agents such as ampicillin, cefpodoxime, and cefotaxime (**Table S1**). ESBL production was confirmed in all the isolates. The 18 isolates were highly diverse, comprising 16 different STs (see **Table S2** and **Table S3**). The *bla*_{CTX-M} genes detected were 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 = 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 (KTa008) carried two copies of the *bla*_{CTX-M-14} gene.

Of the 18 isolates, the genomes of 14 could be completed. These genomes carried 0 to 8 plasmids (see **Table S2** for details). The genomes of the remaining four isolates could not be completed, probably due to the presence of long repeats in the genomes (see **Table S3** for information on the draft assemblies of these genomes).

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

The location and genetic contexts of the *bla*_{CTX-M} genes were determined for the 14 isolates with completed genomes. Of these 14, nine carried *bla*_{CTX-M} genes on chromosomes and five carried *bla*_{CTX-M} genes on plasmids.

In eight of the nine isolates which carried *bla*_{CTX-M} genes on chromosomes, the *bla*_{CTX-M} genes were associated with *ISEcp1* and carried on transposition units (TPU) of different sizes (2,855 bp to 11,093 bp) (**Figure 1**). These TPU were flanked by AT-rich 5-bp target site duplications (TSD). None of the TPU were identical, and the insertion sites of the TPU were different in all cases, indicating that different transposition events contributed to the chromosomal integration of the TPU. Notably, the TPU in KFu023 contained multiple resistance genes, including *bla*_{CTX-M-15}, *aac(6')-Ib-cr*, *bla*_{OXA-1}, and *ΔcatB3*. This seems to highlight the ability of *ISEcp1* to capture sequences of different lengths in different transposition events and thus simultaneously mobilize antibiotic resistance genes of different origins (4). In most cases, these TPU were directly inserted into chromosomes. However, in KKa019, the TPU was part of a 35,625 bp multiresistance region bracketed by two copies of IS26 (annotated as IS15DI in the ISFinder database), and the whole multiresistance region was flanked by 8-bp TSD (CGTTGCCG) in the chromosome (**Figure S1**). KTa008 carried two copies of *bla*_{CTX-M-14}, which were separated by ~1,169 kbp in the chromosome. Analysis of the genetic contexts of these *bla*_{CTX-M-14} genes indicated transposition of an already chromosomally-located TPU and an adjacent region into

another chromosomal location (**Figure S2**). This type of ‘recurrent’ transposition was previously reported in clinical ESBLEC, though the TPU and insertion sites were different (25). We searched for *E. coli* genomes in the public database which carry the same TPU in the same chromosomal location as those identified in the present study. BLASTN searches were performed against the nucleotide collection (nr/nt) database in January 2022 using the TPU and surrounding sequences identified in the present study as query sequences. Three of the nine TPU/insertion site combinations were found in the database, while the remaining six were not found, potentially corresponding to novel integration events (**Table S4**).

In KOr019, *bla*_{CTX-M-2} was associated with *ISCR1* and situated within a complex class 1 integron, which is congruent with previous studies (4, 26). This complex class 1 integron was closely related to a recently reported transposon Tn7337 (**Figure S3**) (27). KOr019 carried the IRt (inverted repeat at *tni* end)-*IS6100*-IRt structure, but this structure was truncated by *IS26* in Tn7337. The 17,747 bp structure from IRI (inverted repeat at *intI1* end) to IRt was inserted into the chromosome of KOr019, creating 5-bp TSD of AAATG. This insertion seems to have been mediated by Tni proteins provided *in trans*, since the *tni* genes in the complex class 1 integron were absent (4).

Genetic contexts of *bla*_{CTX-M} genes on plasmids.

The five completed plasmids with *bla*_{CTX-M} genes belonged to IncI1 (n = 4) and IncB/O/K/Z (n = 1) (**Figure 2a**). IncI1 plasmids were typed as four different STs, namely ST3, ST16, ST113,

and ST167, by IncI1 pMLST (22). TPU were inserted into plasmid backbones in the IncI1/ST3, IncI1/ST16, and IncI1/ST167 plasmids. Interestingly, the IncI1/ST16 and IncI1/ST167 plasmids carried the same TPU (except for insertion of IS1 in the IncI1/ST16 plasmid) inserted into the same location. IncI1/ST167 is a single-locus variant of IncI1/ST16, which indicates the TPU was inserted into the plasmid backbone before the ST diversification. In the IncI1/ST113 plasmid, an IS26- Δ IS10R-*bla*_{CTX-M-8}-IS26 structure was inserted into the plasmid. BLASTN analysis identified IncI1 plasmids belonging to the same IncI1-STs and carrying the same TPU/IS26 structures inserted into the same locations from different countries (e.g., MH847453 from France for *bla*_{CTX-M-1}-carrying IncI1/ST3, CP056650 from the UK for *bla*_{CTX-M-55}-carrying IncI1/ST16, KY964068 from Portugal for *bla*_{CTX-M-8}-carrying IncI1/ST113, CP021208 from China for *bla*_{CTX-M-55}-carrying IncI1/ST167), which indicates circulation of epidemic CTX-M IncI1 plasmids in geographically distant countries. Comparison of IncI1 plasmids revealed the replication region, the leading region, and the transfer regions in the IncI1 reference plasmid R64 were well conserved in all IncI1 plasmids detected in this study, while many genes in the stability region were lacking (**Figure 2b**). In the IncB/O/K/Z plasmid, *bla*_{CTX-M-14} was linked to Δ Tn1721 and a class 1 integron. Similar genetic contexts were previously reported (e.g., JF701188).

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

Among the 53 RefSeq environmental *E. coli* genomes, 20 carried *bla*_{CTX-M}. Sixteen carried *bla*_{CTX-M} on plasmids and four carried *bla*_{CTX-M} on chromosomes (**Table S5**). The four genomes with chromosomal *bla*_{CTX-M} belonged to ST38 (n = 2), ST130 (n = 1) and ST648 (n = 1) and originated from various countries (Japan, New Zealand, Norway, and Switzerland), which indicates diverse *E. coli* isolates carrying chromosomal *bla*_{CTX-M} are also present in environmental waters in other countries. In all four genomes, *bla*_{CTX-M} was associated with TPU ranging from 2,971 bp to 13,552 bp. A TPU containing multiple resistance genes besides *bla*_{CTX-M} and a TPU inserted into a multiresistance region were detected, again highlighting the importance of these mechanisms in the emergence of multidrug resistant *E. coli* in the environment (see **Figure S4** for the analysis of the genetic contexts of *bla*_{CTX-M} in these genomes).

ST38, which was detected in two RefSeq genomes containing chromosomal *bla*_{CTX-M}, was also detected in two isolates with chromosomal *bla*_{CTX-M} sequenced in this study. Interestingly, ST38 was also found to be prevalent in some previous studies reporting chromosomal *bla*_{CTX-M} (6, 28, 29). The reason for the prevalence of ST38 among *E. coli* with chromosomal *bla*_{CTX-M} is unknown. However, multiple integration events seem to have been involved in the acquisition of chromosomal *bla*_{CTX-M} in ST38 (see **Figure 1**, **Figure S1**, and **Figure S4**), which potentially indicates ST38 has genetic mechanisms that can promote chromosomal integration of *bla*_{CTX-M}.

Conclusions.

Here, we demonstrated that the genetic contexts of *bla*_{CTX-M} genes in environmental *E. coli* are highly diverse in terms of the associated mobile elements (various TPU, *ISCR1*, and *IS26*) and integration sites. This study also revealed that the chromosomal locations of *bla*_{CTX-M} genes were unexpectedly frequent in environmental ESBLEC in Japan. Chromosomal integration of *bla*_{CTX-M} might allow *E. coli* to stably maintain *bla*_{CTX-M} in the absence of antibiotic selection pressure in environmental waters, though further studies are needed to confirm this.

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References

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

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

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

311 FIGURES

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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*_{CTX-M} genes are indicated with arrows.