AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Specific $bla_{CTX-M-8}$/IncI1 Plasmid Transfer among Genetically Diverse *Escherichia coli* Isolates between Humans and Chickens

Chihiro Norizuki,[a] Jun-ichi Wachino,[b] Masahiro Suzuki,[c] Kumiko Kawamura,[a] Noriyuki Nagano,[d] Kouji Kimura,[b] Yoshichika Arakawa[b]

Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan[a]; Department of Bacteriology, Nagoya University Graduate School of Medicine, Nagoya, Japan[b]; Laboratory of Bacteriology, Aichi Prefecture Institute of Public Health, Nagoya, Japan[c]; Department of Health and Medical Sciences, Shinshu University Graduate School of Medicine, Matsumoto, Japan[d]

ABSTRACT We investigated the genetic backbones of 14 $bla_{CTX-M-8}$-positive *Escherichia coli* isolates recovered from human stool samples and chicken meat. All isolates carried IncI1 plasmids with $bla_{CTX-M-8}$ ($bla_{CTX-M-8}$/IncI1), and most (9/14) belonged to a specific genetic lineage, namely, plasmid sequence type 113 (pST113). The genetic contexts of the nine $bla_{CTX-M-8}$/IncI1 pST113 plasmids were similar, regardless of the source. These results suggest the probable local transfer of $bla_{CTX-M-8}$/IncI1 between humans and chickens with genetically diverse *E. coli*.

KEYWORDS $bla_{CTX-M-8}$, IncI1 plasmid, *Escherichia* coli, retail chicken meat, human

Escherichia coli isolates harboring CTX-M-type extended-spectrum β-lactamase (ESBL) genes have become a global concern because they are widely disseminated in clinical settings, livestock, healthy humans, companion animals, and wild animals (1). CTX-M-type ESBL-producing *E. coli* isolates in livestock/retail meat need special attention because food contamination could be a major cause of their transfer to humans (2, 3). To assess local transmission of the CTX-M-type ESBL gene between livestock/retail meat and humans in Japan, we analyzed the genetic backbones of CTX-M-8-producing *E. coli*, since its spread is still expected to be limited, at least in Japan (4–6). As described below, our findings suggest the possible horizontal transfer of plasmids of specific genetic lineages bearing the CTX-M-8 β-lactamase gene between humans and chicken meat.

We collected CTX-M-type ESBL-producing *E. coli* from several sources, such as ill patients, healthy people handling food (including employees of retail meat shops and meat producers), and retail foods (chicken meat, beef, and pork) (4, 7, 8). The isolates from ill patients were collected from hospitals spread across Japan, while those from healthy people handling food and retail foods were collected in Aichi Prefecture, Japan (4, 7, 8). Only 14 CTX-M-8-producing *E. coli* isolates were identified. Six were from stool samples from healthy food handlers, and eight were from imported chicken meat from Brazil (Table 1). All isolates were resistant to cefotaxime but susceptible to ceftazidime, imipenem, gentamicin, and fosfomycin (Table 1). We performed whole-genome sequencing (WGS) analysis of 14 CTX-M-8-producing *E. coli* isolates with the MiSeq platform and an A5-miseq assembler to investigate their genetic backbones (9). Multilocus sequence typing (MLST) was performed by transferring the WGS data to the MLST 1.8 server (10), and the presence of antibiotic resistance genes was confirmed by transferring the WGS data to the ResFinder 2.1 server (11). MLST showed highly diverse backbones; 14 isolates were classified into 12 different sequence types (STs), although strain ST131 was found in both human stool samples and chicken meat and ST1144 was

Received 31 March 2017

Accepted manuscript posted online 10 April 2017

Citation Norizuki C, Wachino J-I, Suzuki M, Kawamura K, Nagano N, Kimura K, Arakawa Y. 2017. Specific $bla_{CTX-M-8}$/IncI1 plasmid transfer among genetically diverse *Escherichia coli* isolates between humans and chickens. Antimicrob Agents Chemother 61:e00663-17. https://doi.org/10.1128/AAC.00663-17.

Address correspondence to Jun-ichi Wachino, wachino@med.nagoya-u.ac.jp.

TABLE 1 Characteristics of 14 *E. coli* isolates carrying $bla_{CTX\text{-}M\text{-}8}$[a]

Sample	Source	ST	CTX resistance transfer by conjugation	Plasmid replicon type	No. of plasmids	MIC (μg/ml)								Antimicrobial resistance gene(s)
						CTX	CAZ	IPM	GM	TC	CP	FOM	CIP	
HU23	Human stool sample	131	Yes	IncI1, IncFIB, IncFIC (FII)	3	8	0.5	0.25	0.5	0.5	4	0.5	0.25	$bla_{CTX\text{-}M\text{-}8}$
HU447	Human stool sample	1144	Yes	IncI1, Col156, IncFIC (FII), IncFIB, IncQ1	5	4	2	0.06	1	64	4	0.5	≤0.03	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$, *strA/B*, *sul2*, *tetA*, *dfrA8*
HU476	Human stool sample	1144	Yes	IncI1, Col156, IncFIC (FII), IncFIB, IncQ1	5	8	0.5	0.12	0.5	64	8	0.5	≤0.03	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$, *strA/B*, *sul2*, *tetA*, *dfrA8*
HU485	Human stool sample	23	Yes	IncI1, ColpVC, IncFIB	3	8	0.5	0.25	0.5	1	4	1	≤0.03	$bla_{CTX\text{-}M\text{-}8}$
HU493	Human stool sample	1170	Yes	IncI1, IncFIB, IncFIA, IncFIC (FII)	3	8	1	0.25	1	1	4	1	0.12	$bla_{CTX\text{-}M\text{-}8}$
HU590	Human stool sample	2526	Yes	IncI1, IncFIB (K)	2	8	1	0.25	0.5	64	8	1	0.25	$bla_{CTX\text{-}M\text{-}8}$, *aadA5*, *sul2*, *tetA*, *dfrA17*
CH11	Chicken meat	345	Yes	IncI1, IncFII, IncFIB, IncQ1, IncN	5	8	0.5	0.12	0.5	64	4	0.5	0.25	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$, *aadA1*, *strA/B*, *sul1*, *sul2*, *tetA*, *dfrA1*
CH41	Chicken meat	351	Yes	IncI1, IncQ1, IncFII, IncFIB	3	8	1	0.25	0.5	64	4	0.5	0.25	$bla_{CTX\text{-}M\text{-}8}$, *aadA1*, *strA/B*, *sul1*, *sul2*, *tetA*, *dfrA1*
CH42	Chicken meat	88	Yes	IncI1, IncX1, IncFIC (FII), IncFIB	4	8	1	0.25	0.5	128	8	1	0.25	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$, *tetA*
CH49[b]	Chicken meat	224	Yes	IncI1, IncX4, ColpVC, IncFII, IncY, IncX1, IncFIB	7	8	1	0.25	1	128	64	1	32	$bla_{CTX\text{-}M\text{-}8}$, *aadA1*, *aadA2*, *aph(3′)-Ia*, *cmlA1*, *sul3*, *tetA*, *dfrA12*
CH56	Chicken meat	131	No	IncI1, IncFIB, IncFIC(FII), IncX1	4	8	0.5	0.25	0.5	0.5	4	0.5	0.25	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$
CH110	Chicken meat	4576	Yes	IncI1, IncFII, IncQ1, IncFIB	4	8	0.5	0.25	0.5	32	4	1	≤0.03	$bla_{CTX\text{-}M\text{-}8}$, $bla_{TEM\text{-}1B}$, *aadA1*, *strA/B*, *sul1*, *sul2*, *tetA*, *dfrA1*
CH365	Chicken meat	602	Yes	IncI1, Col156, IncFIB, IncFIC (FII)	4	8	1	0.25	0.5	0.5	4	1	≤0.03	$bla_{CTX\text{-}M\text{-}8}$
CH407	Chicken meat	101	Yes	IncI1, IncFIB, IncFIC(FII)	2	8	0.5	0.12	0.5	1	8	1	0.25	$bla_{CTX\text{-}M\text{-}8}$

[a]Abbreviations: CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; GM, gentamicin; TC, tetracycline; CP, chloramphenicol; FOM, fosfomycin; CIP, ciprofloxacin.
[b]Strain CH49 has amino acid substitutions S83L and D87N in GyrA and S80I in ParC, which confer ciprofloxacin resistance.

TABLE 2 Characteristics of 14 *E. coli* transformants carrying IncI1 plasmids with $bla_{CTX-M-8}$

Transformant	Plasmid replicon type	Plasmid ST	Approximate plasmid size estimated by S1 PFGE	MIC (μg/ml) of cefotaxime	β-Lactamase gene
E. coli(pHU23)	IncI1	pST113	91,831 bp[a]	8	$bla_{CTX-M-8}$
E. coli(pHU447)	IncI1	pST131	90 kb	4	$bla_{CTX-M-8}$
E. coli(pHU476)	IncI1	pST131	91 kb	8	$bla_{CTX-M-8}$
E. coli(pHU485)	IncI1	pST114	82 kb	8	$bla_{CTX-M-8}$
E. coli(pHU493)	IncI1	pST113	94 kb	8	$bla_{CTX-M-8}$
E. coli(pHU590)	IncI1	pST113	88 kb	8	$bla_{CTX-M-8}$
E. coli(pCH11)	IncI1	pST113	101,377 bp[a]	8	$bla_{CTX-M-8}$
E. coli(pCH41)	IncI1	pST113	87 kb	8	$bla_{CTX-M-8}$
E. coli(pCH42)	IncI1	pST113	86 kb	8	$bla_{CTX-M-8}$
E. coli(pCH49)	IncI1	pST113	84 kb	8	$bla_{CTX-M-8}$
E. coli(pCH56)	IncI1	pST113	87 kb	8	$bla_{CTX-M-8}$
E. coli(pCH110)	IncI1	pST235	92 kb	8	$bla_{CTX-M-8}$
E. coli(pCH365)	IncI1	pST132	105 kb	8	$bla_{CTX-M-8}$
E. coli(pCH407)	IncI1	pST113	83 kb	8	$bla_{CTX-M-8}$
E. coli DH10B				0.06	

[a]Plasmid size was determined by WGS analysis, gap-closing PCR, and subsequent Sanger sequencing.

found in stool samples from two different people (Table 1). The susceptibility-testing results and carriage of antibiotic resistance genes were quite consistent (Table 1). The replicon types of plasmids carried by 14 CTX-M-8 producers were confirmed by transferring the WGS data to the PlasmidFinder 1.3 server (12). The numbers of plasmids carried by CTX-M-8 producers were estimated by S1 nuclease pulsed-field gel electrophoresis (PFGE) analysis (13) and simple agarose gel electrophoresis of plasmids extracted with the Plasmid Miniprep System (Promega) (Table 1). All 14 CTX-M-8 producers had IncI1 plasmids, as well as several plasmids with different incompatibility groups (Table 1).

A broth-mating conjugation experiment was performed to transfer the cefotaxime resistance phenotype of 14 CTX-M-8 producers to *E. coli* J53 (azide resistant), and 13 conjugants were selected on Luria-Bertani (LB) agar plates containing sodium azide (150 μg/ml) and cefotaxime (1 μg/ml) (Table 1). Further, plasmids were extracted from 14 CTX-M-8 producers and introduced into the *E. coli* DH10B strain by electroporation. Fourteen cefotaxime-resistant *E. coli* DH10B transformants were selected on LB agar plates containing cefotaxime (1 μg/ml) (Table 2). As expected, $bla_{CTX-M-8}$ was detected in these cefotaxime-resistant conjugants and transformants. The plasmids were extracted from 14 *E. coli* DH10B transformants with the Qiagen Plasmid Midi kit and subjected to PFGE. DNA bands corresponding to the plasmids were extracted and used as a DNA template for WGS analysis as described above. The assembled contigs derived from the plasmids were transferred to the PlasmidFinder 1.3 and ResFinder 2.1 servers to investigate the replicon types and presence of antibiotic resistance genes, respectively (11), and plasmid MLST was performed through the pMLST 1.4 server (12). Although the sizes of the 14 plasmids, which were estimated by S1 nuclease PFGE analysis of 14 cefotaxime-resistant *E. coli* DH10B transformants, varied from 82 to 105 kbp, these plasmids were assigned to the IncI1 group and carried $bla_{CTX-M-8}$ as the only antibiotic resistance gene (Table 2). The 14 IncI1 plasmids were assigned to five plasmid STs (pSTs); 9 were pST113, 1 was pST114, 2 were pST131, 1 was pST132, and 1 was pST235 (newly assigned) (Table 2). IncI1 pST113 plasmids were dominant in *E. coli* isolates from humans (3/6) and retail chicken meat (6/8) (Table 2). These results indicated the possibility that the $bla_{CTX-M-8}$ spread in *E. coli* in Japan was mainly due to the horizontal transfer of IncI1 plasmids belonging to a specific genetic lineage, such as pST113, regardless of their sources, rather than due to the distribution of a clonal *E. coli* strain producing CTX-M-8. To date, CTX-M-8-producing *E. coli* isolates have been found in Germany (14), French Guiana (15), Tunisia (16), Kenya (17), Spain (18), and Brazil (19–21), and IncI1 pST113 plasmids harboring $bla_{CTX-M-8}$ have been reported (22, 23). Preferential carriage of $bla_{CTX-M-8}$/IncI1 pST113 plasmids has also been reported in

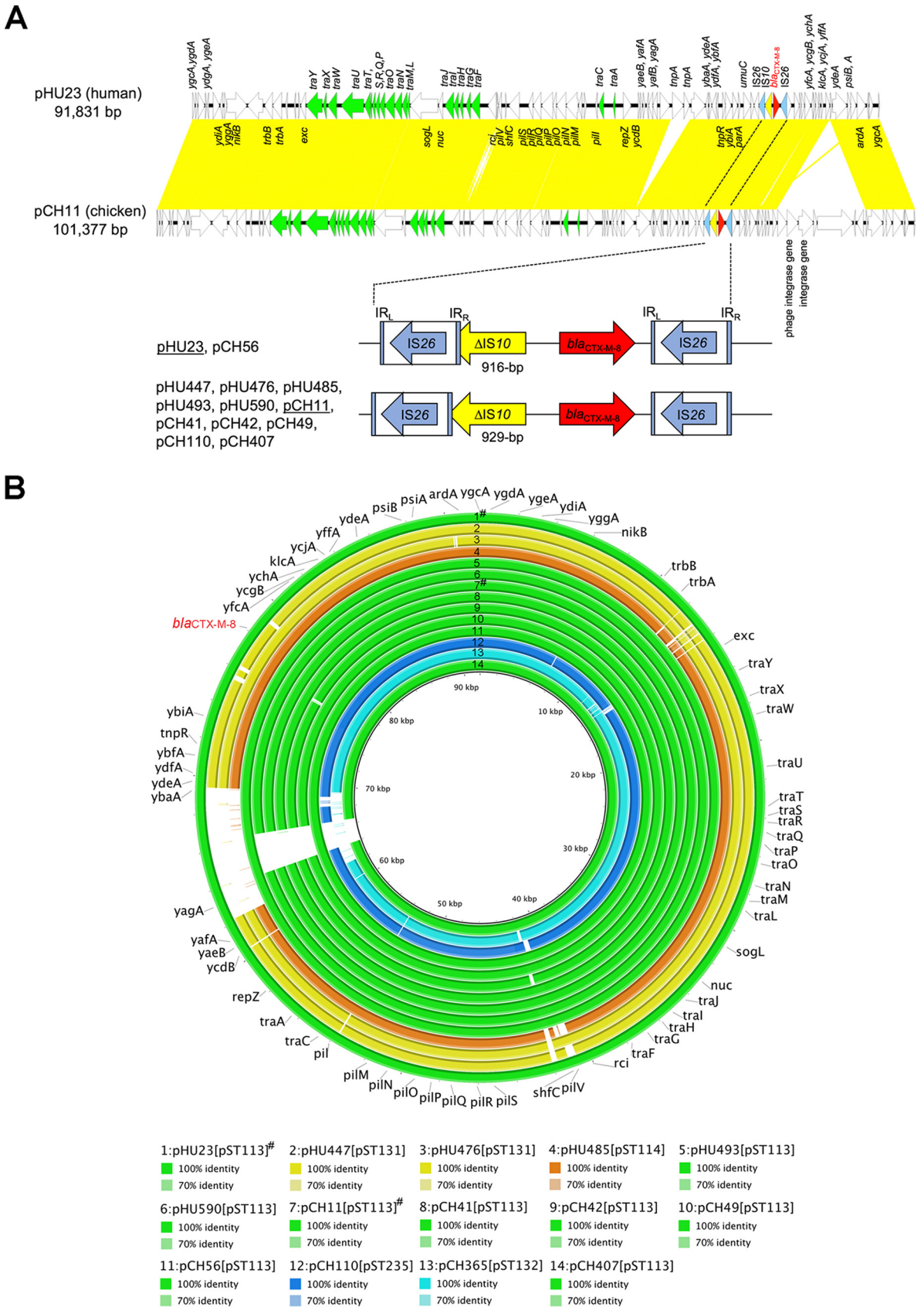

FIG 1 (A) Genetic comparison of the pHU23 plasmid (GenBank accession no. AP017892) with the pCH11 plasmid (GenBank accession no. AP017893). The open reading frames are represented by arrows and color coded according to their functions. $bla_{CTX-M-8}$ is red. Insertion

(Continued on next page)

CTX-M-8-producing *Enterobacteriaceae* isolates, including *E. coli* and *Salmonella* spp. in Germany, whose carriage may be related to contaminated food (14). The carriage of IncI1 plasmids pST114, pST131, and pST132 is lower than that of IncI1 pST113 in this study, and these plasmids were found in CTX-M-8 producers from both humans and poultry in Brazil (22, 23). Worldwide dissemination of the *bla*$_{CTX-M-8}$ gene might also be mediated by specific IncI1 plasmids such as pST113 and less-well-known plasmids pST114, pST131, and pST132.

To further evaluate the genetic backbones of *bla*$_{CTX-M-8}$/IncI1 plasmids from humans and chicken meat, we determined the complete nucleotide sequences of representative *bla*$_{CTX-M-8}$/IncI1 pST113 plasmids, pHU23 from humans, and pCH11 from chicken meat by gap-closing PCR and Sanger sequencing based on the draft sequences of these plasmids. The plasmid sequences were submitted to the Microbial Genome Annotation Pipeline (http://www.migap.org) for annotations. Figure 1A was prepared on the basis of the complete sequences of pHU23 and pCH11 with Easyfig (24). The backbones of the plasmids, including the protein-coding genes *traA* to *traY* responsible for plasmid transfer and the protein-coding genes *pilI* to *pilV* responsible for pilus formation, were identical, and both had no antibiotic resistance gene, except for *bla*$_{CTX-M-8}$ (Fig. 1A). The nucleotide sequence of the pHU23 plasmid showed 97% query coverage and >99% nucleotide identity to that of pCH11. Both plasmids were slightly different in terms of the presence or absence of several putative transposase and integrase genes and hypothetical protein genes (Fig. 1A). Comparison of 14 IncI1/*bla*$_{CTX-M-8}$ plasmids (pHU23 and pCH11 with complete sequences and 12 plasmids with draft sequences) was performed on the basis of the complete sequence of the pHU23 plasmid with BRIG software (25), and nine *bla*$_{CTX-M-8}$/IncI1 pST113 plasmids showed high similarity, regardless of the source (Fig. 1B).

In addition, a common IS*10* element with a partially truncated 3′ end was upstream of the *bla*$_{CTX-M-8}$ gene, although its location slightly differed between pHU23 and pCH11 (Fig. 1A). The *bla*$_{CTX-M-8}$ gene was flanked by two IS*26* elements. Although the WGS analyses of the remaining 12 IncI1 plasmids could not determine the extended genetic region around *bla*$_{CTX-M-8}$, considering the corresponding regions of these plasmids, the genetic region around *bla*$_{CTX-M-8}$ of pCH56 was identical to that of pHU23 with 916-bp ΔIS*10*, while those around *bla*$_{CTX-M-8}$ of pHU447, pHU476, pHU485, pHU493, pHU590, pCH41, pCH42, pCH49, pCH110, and pCH407 were identical to that of pCH11 with 929-bp ΔIS*10* (Fig. 1A). The genetic context around *bla*$_{CTX-M-8}$ in the pCH365 plasmid could not be categorized because the terminal end of the contigs carrying *bla*$_{CTX-M-8}$ neighbored the middle of the IS*10* element. However, the assembled 2,158-bp sequence of the contigs was the same as that of pHU23 and pCH11. Therefore, the DNA sequence around *bla*$_{CTX-M-8}$ showed low diversity among the 14 IncI1 plasmids analyzed, as well as low overall diversity (Fig. 1B), indicating that the *E. coli* isolates from healthy individuals and retail chicken meat had *bla*$_{CTX-M-8}$/IncI1 plasmids with almost the same sequences. These results can potentially explain the possible horizontal transfer of *bla*$_{CTX-M-8}$/IncI1 plasmids with specific genetic lineages between humans and retail chicken meat.

In conclusion, this study is the first to identify and evaluate the genetic relatedness of CTX-M-8-producing *E. coli* derived from different origins (i.e., humans and retail chicken meat), and we revealed the possible horizontal transfer of *bla*$_{CTX-M-8}$/IncI1 plasmids with a specific genetic lineage, such as pST113. In Japan, CTX-M-8-producing *E. coli* has been mainly found in retail chicken meat imported from Brazil (4, 26) but has rarely been found in other sources such as patients in clinical settings and livestock (5,

FIG 1 Legend (Continued)
sequences ΔIS*10* and IS*26* are yellow and blue, respectively. The *tra* region is green. Yellow shading indicates regions with high genetic identity. IR_L, left inverted repeat; IR_R, right inverted repeat. (B) Comparison of 14 *bla*$_{CTX-M-8}$/IncI1plasmids by using BRIG software. The comparison was performed on the basis of the pHU23 plasmid (91,831 bp), whose nucleotide sequences were completely determined. Color coding is based on pST types as follows: green, pST113; yellow, pST131; orange, pST114; blue, pST235; cyan, pST132. Plasmids marked with the symbol # were completely sequenced, while those with draft sequences are not marked.

6, 8). Our findings suggest that carriage of CTX-M-8-producing *E. coli* in humans might be attributed to the horizontal transfer of $bla_{CTX\text{-}M\text{-}8}$/IncI1 harbored by genetically diverse *E. coli* lineages through imported chicken meat. The food handlers analyzed in this study might have acquired CTX-M-8-producing *E. coli* and/or its $bla_{CTX\text{-}M\text{-}8}$/IncI1 plasmids by handling chicken meat. The carriage of antibiotic resistance genes by *E. coli* in retail meat should be regularly and carefully monitored to prevent their further dissemination to humans.

Accession number(s). The complete nucleotide sequences of pHU23 from healthy humans and pCH11 from chicken meat were deposited in the DDBJ database under accession numbers AP017892 and AP017893, respectively.

ACKNOWLEDGMENT

This study was supported by grants from the Food Safety Commission, Cabinet Office, Government of Japan (Research Program for Risk Assessment Study on Food Safety, no. 1504).

REFERENCES

1. Woerther PL, Burdet C, Chachaty E, Andremont A. 2013. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev 26:744–758. https://doi.org/10.1128/CMR.00023-13.
2. Dahms C, Hubner NO, Kossow A, Mellmann A, Dittmann K, Kramer A. 2015. Occurrence of ESBL-producing *Escherichia coli* in livestock and farm workers in Mecklenburg-Western Pomerania, Germany. PLoS One 10:e0143326. https://doi.org/10.1371/journal.pone.0143326.
3. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, Hu J, Lei Y, Li N, Tooming-Klunderud A, Heederik DJ, Fluit AC, Bonten MJ, Willems RJ, de la Cruz F, van Schaik W. 2014. Dissemination of cephalosporin resistance genes between *Escherichia coli* strains from farm animals and humans by specific plasmid lineages. PLoS Genet 10: e1004776. https://doi.org/10.1371/journal.pgen.1004776.
4. Kawamura K, Goto K, Nakane K, Arakawa Y. 2014. Molecular epidemiology of extended-spectrum β-lactamases and *Escherichia coli* isolated from retail foods including chicken meat in Japan. Foodborne Pathog Dis 11:104–110. https://doi.org/10.1089/fpd.2013.1608.
5. Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y. 2009. Change in the prevalence of extended-spectrum-β-lactamase-producing *Escherichia coli* in Japan by clonal spread. J Antimicrob Chemother 63:72–79. https://doi.org/10.1093/jac/dkn463.
6. Hiroi M, Yamazaki F, Harada T, Takahashi N, Iida N, Noda Y, Yagi M, Nishio T, Kanda T, Kawamori F, Sugiyama K, Masuda T, Hara-Kudo Y, Ohashi N. 2012. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. J Vet Med Sci 74:189–195. https://doi.org/10.1292/jvms.11-0372.
7. Nakane K, Kawamura K, Goto K, Arakawa Y. 2016. Long-term colonization by *bla*CTX-M-harboring *Escherichia coli* in healthy Japanese people engaged in food handling. Appl Environ Microbiol 82:1818–1827. https://doi.org/10.1128/AEM.02929-15.
8. Hattori T, Nakane K, Kawamura K. 2011. Multidrug-resistant phenotype and prevalence of CTX-M-27 in extended-spectrum β-lactamase-producing uropathogenic *Escherichia coli*. J Jpn Soc Clin Microbiol 21: 25–34. (In Japanese.)
9. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
10. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.
11. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
12. 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 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
13. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220.
14. Eller C, Leistner R, Guerra B, Fischer J, Wendt C, Rabsch W, Werner G, Pfeifer Y. 2014. Emergence of extended-spectrum β-lactamase (ESBL) CTX-M-8 in Germany. J Antimicrob Chemother 69:562–564. https://doi.org/10.1093/jac/dkt387.
15. Woerther PL, Angebault C, Jacquier H, Clermont O, El Mniai A, Moreau B, Djossou F, Peroz G, Catzeflis F, Denamur E, Andremont A. 2013. Characterization of fecal extended-spectrum-β-lactamase-producing *Escherichia coli* in a remote community during a long time period. Antimicrob Agents Chemother 57:5060–5066. https://doi.org/10.1128/AAC.00848-13.
16. Jouini A, Slama KB, Klibi N, Sallem RB, Estepa V, Vinué L, Saenz Y, Ruiz-Larrea F, Boudabous A, Torres C. 2013. Lineages and virulence gene content among extended-spectrum β-lactamase-producing *Escherichia coli* strains of food origin in Tunisia. J Food Prot 76:323–327. https://doi.org/10.4315/0362-028X.JFP-12-251.
17. Kiiru J, Kariuki S, Goddeeris BM, Butaye P. 2012. Analysis of β-lactamase phenotypes and carriage of selected β-lactamase genes among *Escherichia coli* strains obtained from Kenyan patients during an 18-year period. BMC Microbiol 12:155. https://doi.org/10.1186/1471-2180-12-155.
18. Vinué L, Saenz Y, Martinez S, Somalo S, Moreno MA, Torres C, Zarazaga M. 2009. Prevalence and diversity of extended-spectrum β-lactamases in faecal *Escherichia coli* isolates from healthy humans in Spain. Clin Microbiol Infect 15:954–957. https://doi.org/10.1111/j.1469-0691.2009.02803.x.
19. Bonnet R, Sampaio JL, Labia R, De Champs C, Sirot D, Chanal C, Sirot J. 2000. A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob Agents Chemother 44: 1936–1942. https://doi.org/10.1128/AAC.44.7.1936-1942.2000.
20. Dropa M, Lincopan N, Balsalobre LC, Oliveira DE, Moura RA, Fernandes MR, da Silva QM, Matte GR, Sato MI, Matte MH. 2016. Genetic background of novel sequence types of CTX-M-8- and CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* from public wastewater treatment plants in Sao Paulo, Brazil. Environ Sci Pollut Res Int 23:4953–4958. https://doi.org/10.1007/s11356-016-6079-5.
21. Aizawa J, Neuwirt N, Barbato L, Neves PR, Leigue L, Padilha J, Pestana de Castro AF, Gregory L, Lincopan N. 2014. Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing *Escherichia coli* ST224, ST2179 and ST2308 in buffalo (Bubalus bubalis). J Antimicrob Chemother 69:2866–2869. https://doi.org/10.1093/jac/dku218.
22. Ferreira JC, Penha Filho RA, Andrade LN, Berchieri A, Jr, Darini AL. 2016. Evaluation and characterization of plasmids carrying CTX-M genes in a non-clonal population of multidrug-resistant *Enterobacteriaceae* isolated from poultry in Brazil. Diagn Microbiol Infect Dis 85:444–448. https://doi.org/10.1016/j.diagmicrobio.2016.05.011.

23. Ferreira JC, Penha Filho RA, Andrade LN, Berchieri A, Jr, Darini AL. 2014. IncI1/ST113 and IncI1/ST114 conjugative plasmids carrying *bla*CTX-M-8 in *Escherichia coli* isolated from poultry in Brazil. Diagn Microbiol Infect Dis 80:304–306. https://doi.org/10.1016/j.diagmicrobio.2014.09.012.
24. Sullivan MJ, Petty NK, Beatson SA. 2011. Easyfig: a genome comparison visualizer. Bioinformatics 27:1009–1010. https://doi.org/10.1093/bioinformatics/btr039.
25. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.
26. Shimojima Y, Ida M, Inomata M, Higuchi Y, Takano C, Kawamura M, Konishi N, Hatakeyama K, Nakama A, Kai A. 2011. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from meat. Ann Rep Tokyo Metr Inst Pub Health 62:145–150. (In Japanese.)