〈Original Article〉

# Contamination rate of broad-spectrum cephalosporin-resistant *Enterobacterales* isolated from domestic and imported retail chicken meat in Tokyo and Kanagawa, Japan

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Extended-spectrum cephalosporin-resistant *Enterobacterales* are among the most prevalent antimicrobial-resistant organisms in Gram-negative bacteria, and their spread in the veterinary field is of global concern. We determined the contamination rate of extended-spectrum cephalosporin-resistant *Enterobacterales* in domestic and imported retail chickens in Japan, and examined their antimicrobial resistance and molecular characteristics. Fifty-three domestic and thirty-nine imported retail chickens were purchased, and antimicrobial susceptibility, *β*-lactamase encoding genes, sequence type, and incompatibility type of plasmids were assessed in the isolates. The contamination rates of cefotaxime-resistant *Enterobacterales* isolated from domestic and imported retail chickens were 60.4% and 87.2%, respectively ($p < 0.05$). Approximately 50% of isolates harbored the $bla_{\text{CTX-M}}$ gene, with $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$ groups predominating domestic and imported samples, respectively. The gene for plasmid-mediated AmpC was detected in 34% and 33% of isolates from domestic and imported samples, respectively. Isolates possessing $bla_{\text{CIT}}$ with IncK plasmids were found in the isolates from both sample groups. Additionally, our results indicated that the IncK plasmid had the potential for transconjugation with $bla_{\text{CIT}}$. *Escherichia coli* sequence type (ST) 57 and ST117 were predominant, followed by ST38. The findings of this study suggest that the high contamination

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rate of drug-resistant bacteria in retail chicken meat leads to frequent exposure to these pathogens by the general public in Tokyo and Kanagawa, Japan.

**Key words:** *Escherichia coli*, extended-spectrum *β*-lactamase, plasmid-mediated AmpC *β*-lactamase, plasmid incompatibility type K

## Introduction

Extended-spectrum cephalosporin-resistant *Enterobacterales* are the most prevalent antimicrobial-resistant organisms among Gram-negative bacteria, spreading to both patients and healthy individuals as well as amimals, including companion animals, livestock, and environments[1]. In agricultural and veterinary settings, antibiotics are frequently employed as pharmaceuticals for animals, as growth promoters for livestock. This use may contribute to the spreading of broad-spectrum cephalosporin-resistant *Enterobacterales* in livestock farming environments and the emergence of the bacterial strains carrying genetic determinants of cephalosporin resistance, such as Extended-Spectrum *β*-Lactamases (ESBLs) and plasmid-mediated AmpC (pAmpC) genes. The selection of antimicrobial resistant organisms in livestock and its dissemination in the farms directly impact the contamination of their retail meat. The contamination of ESBLs-producing organisms in retail chicken meat has been investigated in several countries[2–5].

ESBLs and AmpC *β*-lactamase-producing *Escherichia coli* were detected in poultry between 1999 and 2007 in Japan[6, 7]. Hiki *et al.* demonstrated that the restriction of ceftiofur use at the hatchery level has reduced the percentage of broad-spectrum cephalosporin-resistant *E. coli* isolates from broilers since 2012, when the off-label use of ceftiofur in conjunction with *in ovo* vaccination or vaccination of newly hatched chicks was voluntarily discontinued in Japan[8]. However, the influence at the retail level on the contamination rate of extended-spectrum cephalosporin-resistant *Enterobacterales* in domestic chickens has not been evaluated. Additionally, Japan imports chicken from Brazil, the US, Thailand, and China (https://www.alic.go.jp/joho-c/joho05_000073.html). Given that antibiotic use in livestock production processes varies between countries, the molecular epidemiology of the extended-spectrum cephalosporin-resistant *Enterobacterales* between Japan and each country is likely to differ. The harboring and spread of ESBL encoding genes in *Enterobacterales* is depends on the host organism's clone, plasmid incompatibility types, and other factors. However, there are few reports on the differences between isolates from domestic and imported retail chicken meat in these epidemiological and characteristic data.

Considering the possibility that contaminated retail meat may be a source of food-borne antimicrobial resistance, it is important to regularly monitor antibitoic resistance to protect public health. In this study, we assessed the contamination rate of extended-spectrum cephalosporin-re-

sistant *Enterobacterales* in domestic and imported retail chicken, and examined their antimicrobial resistance profiles and genotypic characteristics, such as *β*-lactamase encoding genes, plasmid incompatibility typing, and clonal relationships.

## Materials and Methods

### 1) Isolation from samples, identification, and antimicrobial susceptibility test

Domestic (53 products) and imported (39 products) retail fresh chicken meat samples (34, 3, and 2 samples from Brazil, Thailand, and the US, respectively) were randomly purchased (January 2016–August 2019) from various markets in Tokyo metropolitan and Kanagawa prefecture, Japan. Samples were purchased as a maximum of two different products per market. Ten grams of each retail meat sample were homogenized and were incubated for 18–24 hr in Brain-Heart Infusion culture broth (BD, Japan) supplemented with 2 mg/L cefotaxime (CTX) (Sigma, Japan). Cultured samples were spread on MacConkey agar (Eiken Chemical, Japan) containing 2 mg/L CTX, incubated at 37°C for 18–24 hr. After the incubation, four to six colonies suspected to be *Enterobacterales* were randomly picked up from each meat sample and were subjected to further analysis. *Enterobacterales* isolates were identified by API 20E (bioMerieux, France).

Antimicrobial susceptibility testing was carried out using agar plate dilution methods including ampicillin (ABPC) (Wako, Japan), cefazolin (CEZ) (Sigma, Japan), cefmetazole (CMZ) (Sigma, Japan), ceftazidime (CAZ) (Sigma, Japan), CTX, imipenem (IPM) (Wako, Japan), gentamycin (GM) (Wako, Japan), amikacin (AMK) (Wako, Japan), and ciprofloxacin (CPFX) (Wako, Japan). The concentration ranges of ABPC and AMK were 4–128 mg/L, and those of the remainders were 0.5–16 mg/L. The quality control strain was *E. coli* ATCC 25922. Breakpoints were determined according to the recommendations of the Clinical and Laboratory Standards Institute[9]. An ESBL confirmation was performed by a double-disk synergy test using CTX (BD, Japan), CTX/clavulanate (Eiken Chemical, Japan), CAZ (BD, Japan), and CAZ/clavulanate (Eiken Chemical, Japan).

### 2) DNA extraction and detection of *β*-lactamase encoding genes

A DNA template was obtained using the DNeasy Blood & Tissue Kit (QIAGEN, Germany). The presence of ESBL and pAmpC *β*-lactamase encoding genes were identified through multiplex PCR amplification of $bla_{\text{CTX-M}}$, $bla_{\text{TEM}}$, and $bla_{\text{SHV}}$ genes, and six main groups ($bla_{\text{ACC}}$, $bla_{\text{CIT}}$, $bla_{\text{DHA}}$, $bla_{\text{EBC}}$, $bla_{\text{FOX}}$, and $bla_{\text{MOX}}$) of pAmpC-type genes, as described previously[10, 11]. In addition, if the strain tested positive for the $bla_{\text{CTX-M}}$ gene, the CTX-M groups (-1, -2, -8/25, and -9) genes of the strain were classified, as described previously[12]. PCR was performed with Takara *Ex taq* (Takara Bio, Japan) to detect these genes. All PCR amplicons were verified by gel electrophoresis on 1.8% SeaKem LE agarose (Takara Bio, Japan).

**3) Multi-locus sequence typing and phylogenetic grouping of *E. coli* strains**

Multi-locus sequence typing (MLST) was performed in accordance with the Achtman scheme using seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*)[13]. The MLST procedure, including allelic type and sequence type (ST) assignment methods, was conducted according to the protocol described on the website (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). A total of 49 *E. coli* strains, randomly selected from isolates, were tested. Among the novel STs found in this study, the single-locus variants of known ST complexes were considered to belong to the previously identified ST complexes. *E. coli* isolates were also analyzed to determine four major phylogenetic groups (A, B1, B2, and D) using a triplex PCR assay of *chuA*, *yjaA*, and *tspE4.C2*, as described previously[14].

**4) Plasmid incompatibility typing**

PCR-based replicon typing was performed on all strains, as described previously[15]. Eighteen primer pairs targeting the FIA, FIB, FIC, HI1, HI2, I1-I$\gamma$, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII replicons were used in multiplex and simplex PCR reactions.

**5) Transconjugation testing**

The transmissibility test was performed the 19 IncK and $bla_{CIT}$-positive isolates. An *E. coli* DH5$\alpha$ strain that was resistant to rifampicin (generated by our laboratory) was used as the recipient. The donor-to-recipient ratio was 1 : 4, and broth mating was incubated at 37°C for 20 hr. Transconjugants were selected on modified Drigalski agar (Eiken Chemical, Japan) supplemented with 50 mg/L rifampicin (Wako, Japan), and 2 mg/L CTX. The transconjugants obtained were subjected to antimicrobial susceptibility testing as used CMZ, CAZ, CPFX, and GM. Additionally, the presence of *bla* genes and the typing including plasmid incompatibility in the transconjugants ware confirmed by PCR.

**6) Statistical analysis**

The contamination rate of the sample and antimicrobial resistance rate of the isolates were compared between domestic and imported retail chicken using the chi-square test and Fisher's exact test. A p-value of $< 0.05$ was considered to be a statistically significant. All statistical calculations were performed using R (version 3.2.1) and R Studio (version 1.0.153).

## Results

**1) Contamination rate and bacterial characterization of isolates**

The contamination rates of CTX-resistant *Enterobacterales* isolated from domestic and imported retail chicken meat were 60.4% (32/53) and 87.2% (34/39), respectively ($p < 0.05$). A

total of 41 and 64 isolates were obtained, with an average of 1.28 and 1.88 isolates per sample from domestic and imported samples, respectively. Of the 105 isolates, 92 were identified as *E. coli*, and the remaining isolates were identified as 8 species, as shown in Table 1. There were 24 and 41 positive strain isolates from domestic and imported samples, respectively, in the ESBL screening test. Ninety-two *E. coli* isolates were further analyzed for distribution of CTX-M and pAmpC type $\beta$-lactamase encoding genes, MLST, phylogenetic grouping, and plasmid incompatibility typing.

The resistance rate of antibiotics among the isolates from domestic and imported samples were 100% vs. 100% for ABPC, 35.6% vs. 35.9% for CMZ ($p = 1$), 100% vs. 100% for CTX, 61.0% vs. 45.3% for CAZ ($p = 0.1299$), 8.8% vs. 11.9% for CFPM ($p = 1$), 0% vs. 0% for IPM, 29.3% vs. 31.3% for CPFX ($p = 1$), 19.5% vs. 54.7% for GM ($p < 0.05$), and 0% vs. 0% for AMK, as shown in Table 2.

**Table 1. Contamination rates and bacterial isolates identified from each sample**

| | Domestic | Imported |
|---|---|---|
| Contamination rate (Number of contaminated samples/Total sample) | 60.4% (32/53) | 87.2% (34/39) |
| Number of isolates from each sample | 41 | 64 |
| Distribution for identified isolates | | |
| *Escherichia coli* | 37 | 55 |
| *Escherichia fergusonii* | — | 1 |
| *Enterobacter cloacae* | 1 | — |
| *Enterobacter intermedius* | — | 2 |
| *Citrobacter freundii* | — | 1 |
| *Klebsiella pneumoniae* | 1 | — |
| *Proteus mirabilis* | — | 4 |
| *Salmonella* sp. | 1 | 1 |
| *Serratia liquefaciens* | 1 | — |
| Number of ESBL screening test-positive isolates | 24 | 41 |

— : The em dash represents no isolate collected.

**Table 2. Comparison of antimicrobial susceptibility profiling of 105 isolates between domestic and imported samples**

| | Susceptible (%) | | Non-susceptible (%) | |
|---|---|---|---|---|
| | Domestic | Imported | Domestic | Imported |
| Ampicillin | 0 (0) | 0 (0) | 41 (100) | 64 (100) |
| Cefmetazole | 26 (63.4) | 41 (64.1) | 15 (36.6) | 23 (35.9) |
| Cefotaxime | 0 (0) | 0 (0) | 41 (100) | 64 (100) |
| Ceftazidime | 16 (30.0) | 35 (54.7) | 25 (70.0) | 29 (45.3) |
| Cefepime | 31 (91.2) | 57 (89.1) | 3 (8.8) | 7 (10.9) |
| Imipenem | 41 (100) | 64 (100) | 0 (0) | 0 (0) |
| Ciprofloxacin | 29 (91.2) | 44 (88.1) | 12 (8.8) | 20 (11.9) |
| Gentamicin | 33 (80.5) | 29 (45.3) | 8 (19.5) | 35 (54.7) |
| Amikacin | 41 (100) | 64 (100) | 0 (0) | 0 (0) |

**2) Distribution of *β*-lactamase encoding genes detected in isolates**

Table 3A shows the number of *bla* genes detected in isolates. Approximately 50% of isolates from domestic and imported chickens possessed the $bla_{\text{CTX-M}}$ type ESBL genes, and $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$ were detected in 40 isolates and one isolate, respectively. It is worth noting that $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$ were included in non-ESBL. In the classification of the $bla_{\text{CTX-M}}$ grouping, the proportion of $bla_{\text{CTX-M-1}}$ group genes was higher in the domestic (13/41, 32%) than in the imported samples (3/64, 5%) ($p < 0.05$). The genes of pAmpC were detected at a rate of 34% and 33% in isolates from domestic and imported samples, respectively, and were classified as belonging to the $bla_{\text{CIT}}$ type. The distribution of isolates possessing two or more *β*-lactamase is shown in Table 3B. Two isolates harboring $bla_{\text{CTX-M}}$ and $bla_{\text{CIT}}$ were isolated from imported samples and identified as *E. coli* and *Proteus mirabilis*.

**3) Multi-locus sequence typing and phylogenetic grouping of *E. coli***

Of the 49 strains of *E. coli*, 34 strains were examined to determine sequence types. The remaining 15 strains had two or more different alleles in the housekeeping genes. As a result, *E. coli* ST57 and ST117 were the most common sequence types with 6 and 6 strains, respectively, followed by *E. coli* ST38, and ST770 (Fig. 1A). All the strains assigned to these sequence types were isolated from imported samples. On the other hand, the sequence types of *E. coli* isolates from domestic samples were diverse, and categorized into 15 types of STs.

Fig. 1B indicates the relationship between the STs and the *β*-lactamases determined in *E. coli* isolates. The isolates harboring the $bla_{\text{CTX-M-1}}$ group of ESBL genes—which were predominantly detected in isolates from domestic samples—were classified into ST10, ST69, ST355, and

**Table 3. Distribution of *β*-lactamase genotypes of 105 isolates between domestic and imported samples**

A

| *β*-lactamases genes | Number of positive isolates (%) | |
|---|---|---|
| | Domestic | Imported |
| ESBL | | |
| $bla_{\text{CTX-M-1}}$ group | 13 (32) | 3 (5) |
| $bla_{\text{CTX-M-2}}$ group | 4 (10) | 28 (44) |
| $bla_{\text{CTX-M-8}}$ group | 0 (0) | 1 (3) |
| $bla_{\text{CTX-M-9}}$ group | 7 (17) | 0 (0) |
| $bla_{\text{TEM}}$ [a] | 20 (49) | 20 (31) |
| $bla_{\text{SHV}}$ [a] | 0 (0) | 1 (2) |
| pAmpC | | |
| $bla_{\text{CIT}}$ | 14 (34) | 21 (33) |
| Total isolates | 41 | 64 |

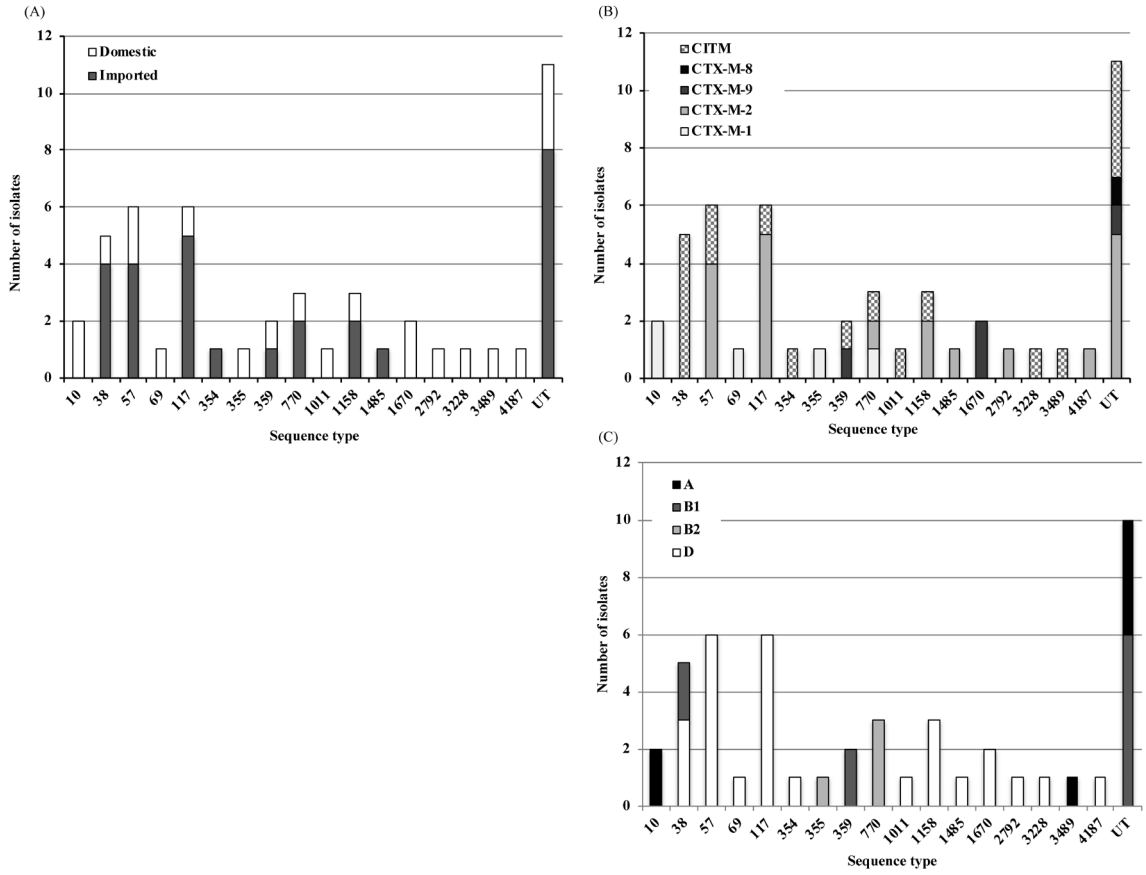
B

| *β*-lactamases genes | Number of positive isolates | |
|---|---|---|
| | Domestic | Imported |
| ESBL | | |
| $bla_{\text{CTX-M-1}}$ group + $bla_{\text{TEM}}$ [a] | 10 | 3 |
| $bla_{\text{CTX-M-2}}$ group + $bla_{\text{TEM}}$ [a] | 1 | 9 |
| $bla_{\text{CTX-M-9}}$ group + $bla_{\text{TEM}}$ [a] | 4 | 0 |
| ESBL + pAmpC | | |
| $bla_{\text{CTX-M-2}}$ group + $bla_{\text{CIT}}$ | 0 | 2 [b] |
| $bla_{\text{TEM}}$ [a] + $bla_{\text{CIT}}$ | 5 | 4 [b] |

A: The number of isolates harbored single *β*-lactamase gene detections from each strain, B: The number of isolates harbored two or more combined *β*-lactamase gene detections from each strain.

*a*: $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$ were included in non-ESBL, b: One isolate harbored $bla_{\text{CTX-M-2}}$, $bla_{\text{TEM}}$, and $bla_{\text{CIT}}$.

**Fig. 1. Distribution of Multi-locus Sequence Typing (MLST) of *E. coli* Isolates**




All graphs show MLST with (A): sample source, (B): determinate *β*-lactamase encoding genes, and (C): phylogenetic grouping.

ST770. Then, the isolates harboring the $bla_{CIT}$ type of pAmpC genes were extensively classified into 10 sequence types. *E. coli* ST38, possessing *β*-lactamase, included only the $bla_{CIT}$ type of pAmpC gene.

The ST, except for ST38, was recognized in each phylogenetic group, as shown in Fig. 1C. In both domestic and imported samples, the dominant phylogenetic group of *E. coli* isolates was group D, followed by group A. In the domestic sample, the proportion of group D isolates was over 50%, whereas in the imported samples, the proportions of group A and group D of the isolates were almost equal at 40%.

**4) Plasmid incompatibility typing and *β*-lactamase in *E. coli***

The distribution of the plasmid Inc type in *E. coli* was shown in Table 4. The plasmid type IncFIB was the most dominant among all *β*-lactamase groups possessing isolates. The types of IncHI1, IncN, IncP, and IncA/C were found only in isolates from domestic samples. On the other hand, there were no Inc types found in isolates from imported samples. In comparison with

**Table 4. Number of positive isolates including plasmid incompatibility type and *β*-lactamase genes**

| *β*-lactamase genes | Domestic or Imported | Number of isolates distributed for each replicon type from domestic and imported samples | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HI1 | HI2 | I1-I*γ* | X | L/M | N | FIA | FIB | W | Y | P | FIC | A/C | T | FIIAs | Frep | K | B/O |
| $bla_{CTX\text{-}M\text{-}1}$ group | Domestic | — | — | 4 | — | — | 3 | 1 | 9 | — | — | 1 | — | — | — | — | 11 | 1 | 4 |
| | Imported | — | — | 0 | — | — | 0 | 0 | 2 | — | — | 0 | — | — | — | — | 2 | 0 | 0 |
| $bla_{CTX\text{-}M\text{-}2}$ group | Domestic | — | — | 2 | — | — | — | 1 | 2 | — | — | — | — | — | — | — | 4 | — | 0 |
| | Imported | — | — | 0 | — | — | — | 0 | 23 | — | — | — | — | — | — | — | 21 | — | 3 |
| $bla_{CTX\text{-}M\text{-}8}$ group | Domestic | — | — | 0 | — | — | — | — | 0 | — | — | — | — | — | — | — | 1 | — | — |
| | Imported | — | — | 1 | — | — | — | — | 1 | — | — | — | — | — | — | — | 0 | — | — |
| $bla_{CTX\text{-}M\text{-}9}$ group | Domestic | — | — | — | — | — | — | 2 | 7 | — | — | — | 1 | — | — | — | 6 | — | 1 |
| | Imported | — | — | — | — | — | — | 0 | 0 | — | — | — | 0 | — | — | — | 0 | — | 0 |
| $bla_{CIT}$ | Domestic | 1 | — | 5 | — | — | — | 2 | 10 | — | 2 | 1 | 0 | 3 | — | — | 10 | 9 | 5 |
| | Imported | 0 | — | 3 | — | — | — | 2 | 16 | — | 0 | 0 | 1 | 0 | — | — | 11 | 10 | 5 |

— : The em dash represents no isolate collected.

*β*-lactamase types, the diversity of the Inc type in isolates harboring $bla_{CTX\text{-}M}$ was higher than that in isolates harboring pAmpC genes. The isolates involved in IncN were found only in those harboring $bla_{CTX\text{-}M}$, which was classified as the $bla_{CTX\text{-}M\text{-}1}$ group. On the other hand, IncFIB and IncK were simultaneously found in isolates harboring the $bla_{CIT}$ type pAmpC gene from both domestic and imported samples.

**5) Ability of Transconjugation of IncK plasmid in *E. coli***

The IncK-positive and $bla_{CIT}$-positive *E. coli* isolates from domestic and imported samples demonstrated transtcojugation ability in 8/9 (88.9%) and 9/10 (90.0%) cases, respectively. The antimicrobial susceptibility of transconjugants was exhibited to be resistant for CMZ and CAZ. The *β*-lactamases genes transferred in the transconjugants were all detected as $bla_{CIT}$. Additionally, plasmid incompatibility was detected IncK in all isolates. The frequency of the transconjugation did not differ between isolates from domestic ($3.07\times10^{-3}-2.78\times10^{-8}$) and imported ($1.77\times10^{-4}-5.34\times10^{-8}$) samples (Table 5).

## Discussion

In the present study, we aimed to investigate the prevalence and molecular epidemiology of extended-spectrum cephalosporin-resistant *Enterobacterales* in retail chicken meat in the Tokyo metropolitan and Kanagawa prefecture regions of Japan. The contamination rate of the strain was found to between 60–90% in both domestic and imported retail chickens, higher than previously reported[16]. Such differences in contamination rates may be due to differences in years, regions, and sampling sizes of the products examined. Hiki *et al.* reported a decrease in the cephalosporin resistance rate in *E. coli* isolated from healthy broilers at farms after a voluntary withdrawal of ceftiofur[8]. However, both the study by Kawamura *et al.* and our own study indicated that the de-

**Table 5. The ability of transconjugation for 19 IncK and *bla*$_{CIT}$-positive *E. coli* isolates**

| Strain [a] | Antimicrobial susceptibility [b] (Donor/Tc) | | | | *β*-lactamase genes [c] | | Plasmid inc typing | | Frequency [d] |
|---|---|---|---|---|---|---|---|---|---|
| | CMZ | CAZ | CPFX | GM | Donor | Tc | Donor | Tc | |
| D-1 | I/- | I/- | S/- | S/- | CIT | — | A/C K | — | — |
| D-2 | I/S | R/R | S/S | S/S | CIT | CIT | I1-I*γ* FIB Y K | I1-I*γ* FIB Y K | $2.78\times10^{-8}$ |
| D-3 | S/R | R/R | S/S | S/S | CIT T | CIT | I1-I*γ* FIA FIB F K | K | $6.67\times10^{-5}$ |
| D-4 | R/R | R/R | S/S | S/S | CIT T | CIT | HI1 FIB F K | K | $2.93\times10^{-4}$ |
| D-5 | I/R | R/R | R/S | S/S | CIT T | CIT T | FIB Y F K | K | $3.74\times10^{-5}$ |
| D-6 | I/R | R/R | S/S | R/S | CIT T | CIT | FIB A/C F K | K | $4.20\times10^{-7}$ |
| D-7 | I/R | R/R | S/S | R/R | CIT | CIT | FIB F K B/O | K B/O | $3.69\times10^{-3}$ |
| D-8 | I/R | R/R | S/S | R/R | CIT | CIT | P FIB F K B/O | P K B/O | $3.07\times10^{-3}$ |
| D-9 | I/I | R/R | S/S | R/I | CIT | CIT | I1-I*γ* FIB F K B/O | I1-I*γ* K B/O | $1.36\times10^{-6}$ |
| I-1 | I/S | I/R | S/S | R/S | CIT | CIT | FIB F K | K | $1.84\times10^{-6}$ |
| I-2 | R/R | R/R | R/S | R/S | CIT T | CIT | I1-I*γ* FIB F K | K | $1.77\times10^{-4}$ |
| I-3 | S/R | I/R | R/S | S/S | CIT | CIT | I1-I*γ* FIB K | K | $4.15\times10^{-4}$ |
| I-4 | I/R | R/R | R/S | S/S | CIT | CIT | FIB F K | K | $7.92\times10^{-5}$ |
| I-5 | I/R | I/R | S/S | S/S | CIT | CIT | K | K | $3.67\times10^{-6}$ |
| I-6 | I/S | I/R | S/S | S/S | CIT | CIT | FIB K | K | $4.73\times10^{-7}$ |
| I-7 | I/S | I/R | S/S | S/S | CIT | CIT | FIB K | K | $5.34\times10^{-8}$ |
| I-8 | I/- | R/- | R/- | R/- | CIT T | — | FIA FIB F K | — | — |
| I-9 | I/R | R/R | S/S | R/R | CIT | CIT | FIB F K B/O | K B/O | $1.68\times10^{-7}$ |
| I-10 | I/R | R/R | S/S | S/R | CIT | CIT | FIB F K B/O | FIB K B/O | $2.93\times10^{-6}$ |

*a*: Tested strains from domestic samples (D), and from imported samples (I).
*b*: S, susceptible; I, intermediate; R, resistant. CMZ, cefmetazole; CAZ, ceftazidime; CPFX, ciprofloxacin; GEN, gentamicin. The Clinical and Laboratory Standards Institute breakpoints were used to interpret the susceptibility results.
*c*: CIT, *bla*$_{CIT}$ (One of the plasmid-mediated AmpC group, including *bla*$_{CMY-2,}$ etc.); T, *bla*$_{TEM.}$
*d:* Frequency was calculated as the number of colonies of transconjugant divided by the recipient. —：The em dash represents no obtained the transconjugant.

crease in resistance rate at both the broiler and farm environment was not accompanied by a decrease in the contamination rate of extended-spectrum cephalosporin-resistant bacteria in retail meat[16]. This suggests that the prevalence of retail chicken meat contaminated with drug-resistant bacteria is strongly influenced not only by the colonization of the bacteria in the poultry intestine, but also by contamination at the slaughterhouse and other factors. Furthermore, our results indicate that the proportion of the extended-spectrum cephalosporins resistance mechanism mediated by ESBL and pAmpC *β*-lactamases among *Enterobacterales* isolates was similar between domestic and imported retail chicken meat in the Tokyo metropolitan and Kanagawa prefecture regions of Japan.

Initially, there was a marked difference in the proportion of *bla*$_{CTX-M}$ group classification between domestic and imported samples. Kameyama *et al.* conducted an investigation into the epidemiology of broad-spectrum cephalosporin-resistant *E. coli* from various broiler farms and found that the *bla*$_{CTX-M-1}$ group, which comprised of *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-15}$, and *bla*$_{CTX-M-55}$, was dominant in Japan[17]. Additionally, the dissemination of CTX-M-15 ESBL- and CTX-M-55 ESBL-producing *E. coli* has been reported through surveillance on farms in Europe and Southeast Asia[18, 19]. Notably, the *bla*$_{CTX-M-55}$ ESBL gene is frequently detected in livestock, farms, and

meat production in Southeast Asia and East Asia[20–22]. Therefore, it is expected that the types of $bla_{\text{CTX-M-1}}$ group ESBL genes present among the isolates from domestic samples in this study will be designated as $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-55}}$. However, this is unclear in this study because we did not determine as far as sequencing the $bla_{\text{CTX-M}}$ gene. From the point of view of incompatibility typing, isolates possessing $bla_{\text{CTX-M}}$, which is associated with the dominant CTX-resistant factor, generally carry the IncFIB type plasmid. IncI, IncN, and IncFIA were identified only in the isolates from domestic samples (Table 4). These varieties of Inc types were consistent with the $bla_{\text{CTX-M}}$ coding plasmid found in the isolates from patients in Japan[23]. On the other hand, of course, it is difficult to refer to a direct relationship between meat and patient derived $bla_{\text{CTX-M}}$ based on incompatibility type concordance alone, and analysis of the surrounding structure of the *bla* genes and comparison with clinical strains is necessary even for the isolates.

In this study, the majority of retail chicken meat was sourced from Brazil, and the ESBL coding genes carried by the isolates were predominantly from the $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ groups, with high GM and CPFX resistance rates, which is in accordance with previous studies[16]. It is reported that $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ encoding integrons possessing *Enterobacterales* have spread in Brazil[24]. Moreover, Norizuki *et al.* also demonstrated that the $bla_{\text{CTX-M-8}}$-possessing *E. coli* was also detected in healthy humans, and the region surrounding $bla_{\text{CTX-M-8}}$ was similar to the component genes found in isolates from imported retail meat samples[25]. These findings strongly suggest that antimicrobial resistant genes and/or cassettes may transfer $bla_{\text{CTX-M-8}}$ from contaminated bacteria in retail meat to intestinal bacteria in human through ingestion. If the isolates in this study also harbor GM or CPFX resistance genes within their integron structure, there is a concern that exposure to imported retail chicken meat may lead to dissemination of multidrug-resistant strains to the general public. Indeed, fecal carriage of ESBL-producing *Enterobacterales* in healthy adults is on the rise[26]. Sequence types 38, 57, and 117 of *E. coli* contained $bla_{\text{CTX-M-2}}$ group ESBL genes, indicating that the *E. coli* of these sequence types harboring $bla_{\text{CTX-M-2}}$ group were colonized in foreign broiler farms. In particularly, *E. coli* ST38 and ST117 isolates were associated with humans[27–28]. In light of this, attention should not only be paid to the resistance gene cassette, but also to the direct transfer of the strain as a factor in the entry of resistance genes in meat into the human intestinal tract.

Secondly, IncK-positive strains in both samples simultaneously carried $bla_{\text{CIT}}$. Although it has been reported that *E. coli* isolates possessing IncK with pAmpC $\beta$-lactamase were present in the surveillance of Japanese broilers, the report did not provide detailed information about the characteristics of the strains because it only obtained a few isolates carrying IncK with the pAmpC $\beta$-lactamase encoding gene. Thus, this present report is the first to document the epidemiological isolation of *E. coli* carrying IncK with $bla_{\text{CIT}}$ from retail chicken meat in Japan. Furthermore, we also demonstrated that the plasmid classified IncK has a high potential for transconjugation (17/19, 89.5%) with $bla_{\text{CIT}}$ (Table 5). Indeed, Hiki *et al.* found that 41.9% (13/31) of

transconjugants possessed *bla*$_{\text{CMY-2}}$ carrying plasmids[29]. However, they have not clarified even the Inc typing of the plasmid possessed by the isolates. On the other hand, Seiffert *et al.* determined the complete sequence of the IncK plasmid in *E. coli* harboring *bla*$_{\text{CMY-2/4}}$ and reported that the isolates from poultry, chicken and humans share the plasmid in Switzerland[30]. Consequently, in this study, the diversity of *E. coli* sequence types carrying *bla*$_{\text{CIT}}$ may be due to the fact that the IncK plasmid has transconjugation ability and its horizontal transfer occurs from one strain to another sequence type strain. Unfortunately, the reason why strains harboring *bla*$_{\text{CMY-2}}$ are not so widespread among healthy humans and patients in Japan is currently unknown.

Our study has several limitations. First, the locations where retail chicken meat was purchased were regionally restricted. Second, it is unclear whether the imported chicken meat samples were processed at a poultry slaughterhouse or supermarket. Then, if the imported chicken meats collected in this study was processed domestically, it cannot be ruled out that the isolates on the samples may be contaminants from other sources. However, the high contamination of the strain isolated from retail chicken meat in Japan raise concerns about antimicrobial resistance and the need for continued surveillance of antimicrobial resistance. Additionally, it is anticipated that the potential influence of human intestinal colonization through ingestion of retail meat will be directly effective against community dwellers, as the retail meat samples in this study were available to consumers in the community. Indeed, in exploring the possibility of transfer of resistance genes from retail meat, continuous surveillance of broad-spectrum cephalosporin-resistant *Enterobacterales* in the healthy carriers is clearly necessary.

## Conclusion

In this study, retail chicken meats was found to be highly contaminated with extended-spectrum cephalosporin-resistant *Enterobacterales*, with a higher contamination rate in imported samples. The proportion of *β*-lactamase genes in isolates was comparable between ESBL and pAmpC encoding genes. However, the proportion of *bla*$_{\text{CTX-M}}$ group genes harbored the isolates differed between domestic and imported samples, with *bla*$_{\text{CTX-M-1}}$ group and *bla*$_{\text{CTX-M-2}}$ group being predominant in domestic and imported samples, respectively. The pAmpC gene, classified as *bla*$_{\text{CIT,}}$ was detected in approximately 35% of isolates. The IncK plasmid was found to have the ability to transconjugate in a high percentage of different ST isolates. This high-level of drug-resistant bacterial contamination of retail chicken meat presents a risk of frequent contact with the general public in Tokyo and Kanagawa, Japan.

## Declarations

### Availability of data and materials

All datasets on which the conclusions of the manuscript rely are presented in the paper.

### Competing interests

We have no conflict of interest to declare.

### Author's contribution

Hasunuma, Y designed the experiments and wrote the manuscript. Hiyoshi, N. carried out experiments, among which she mainly led the plasmid transconjugation and molecular epidemiological analysis. Tanaka, M. collected strains and conducted antimicrobial susceptibility testing. Tokuoka, Y is organizing our research. All of the authors read and approved the final manuscript.

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