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CTX-M-14 Type β -Lactamase Producing *Salmonella enterica* Seroovar Enteritidis Isolated from Imported Chicken Meat

Yuko Matsumoto*, Harue Kitazume, Mikiko Yamada, Yukiko Ishiguro,
Tetsunori Muto, Hidemasa Izumiya¹ and Haruo Watanabe¹

Yokohama City Institute of Health, Yokohama 235-0012, and
¹National Institute of Infectious Diseases, Tokyo 162-8640, Japan

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Salmonella continues to be one of the main causative agents of food poisoning. Chicken meat is one of the foods most frequently contaminated by this agent. The Yokohama City

Institute of Health has been monitoring *Salmonella* contamination in domestically produced chicken meat and imported chicken meat marketed in Yokohama since 1982 and since 1999, respectively.

From 1999 to 2005, 48 *Salmonella enterica* serovar Enteritidis (SE) strains were isolated from 152 chicken meat specimens (isolation rate, 31.6%). On account of the recent surge of drug-resistant *Salmonella*, we examined the isolates

*Corresponding author: Mailing address: Yokohama City Institute of Health, 1-2-17 Takigashira, Isogo-ku, Yokohama, Kanagawa 235-0012, Japan. Tel: +81-45-754-9803, Fax: +81-45-754-2210, E-mail: yu02-matsumoto@city.yokohama.jp

Table 1. MICs of various antimicrobials for *Salmonella enterica* serovar Enteritidis from chicken meat isolates and their transconjugants

Strain	Species	MIC ($\mu\text{g/mL}$) ¹⁾															
		ABPC	CET	CXM	CTX	CTRX	CPZ	AZT	CAZ	CFX	CTT	ESBL test		NA	OFLX	CPFX	LVFX
												CTX	CTL				
Y18809	<i>S. Enteritidis</i>	>256	>256	>256	256	>256	>256	8	4	4	0.094	>16	0.094	>256	2	0.25	0.5
DH10B	<i>E. coli</i>	4	8	6	0.094	0.125	0.38	0.125	0.5	12	0.5	NT	NT	2	0.032	0.004	0.008
Y21134 ²⁾	<i>E. coli</i>	>256	>256	>256	>256	>256	256	6	3	12	0.5	>16	0.064	2	0.032	0.004	0.008
Y18808 ³⁾	<i>S. Enteritidis</i>	1.5	3	3	0.125	0.094	1	0.064	0.38	2	0.094	NT	NT	>256	2	0.25	0.5
Y21135	<i>S. Enteritidis</i>	>256	>256	>256	>256	>256	>256	6	4	2	0.125	>16	0.094	>256	2	0.25	0.5

¹⁾: Determined by Etest.²⁾: DH10B transformant of ps1; Donor of transconjugation.³⁾: Recipient of transconjugation.

ABPC, ampicillin; CET, cephalothin; CXM, cefuroxime; CTX, cefotaxime; CTRX, ceftriaxone; CPZ, cefoperazone; AZT, aztreonam; CAZ, ceftazidime; CFX, cefoxitin; CTT, cefotetan; CTL, cefotaxime plus clavulanic acid; NA, nalidixic acid; OFLX, ofloxacin; CPFX, ciprofloxacin; LVFX, levofloxacin; NT, not tested.

for resistance against 11 antibiotics (ampicillin, streptomycin, tetracycline, ciprofloxacin, kanamycin, cefotaxime, chloramphenicol, trimethoprim/sulphamethoxazole, gentamicin, nalidixic acid and fosfomycin). Drug sensitivity was measured using a disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) standard and employing Sensidisc (Nippon Becton Dickinson, Tokyo, Japan) (1,2). A strain that was isolated in 2004 from chicken meat imported from China and sold by a retailer in Yokohama (strain Y18809) was found to be resistant to ampicillin, cefotaxime and nalidixic acid.

As food-derived SE resistant to a third generation cephalosporin had not been yet reported, we conducted biological and genetic analyses of the strain SE Y18809. Antibiotic sensitivity testing by Etest (AB biodisk, Solna, Sweden) revealed that this strain was resistant to ampicillin, cephalothin, cefuroxime, cefotaxime, ceftriaxone and cefoperazone, and sensitive to cefotetan. It also showed reduced sensitivity to aztreonam, ceftazidime and cefoxitin. Among quinolones, it was resistant to nalidixic acid and showed reduced sensitivity to ofloxacin, ciprofloxacin and levofloxacin (Table 1). In the Etest ESBL (extended-spectrum β -lactamase), resistance to cefotaxime and ceftazidime was inhibited by clavulanic acid, indicating that the strain was an ESBL producer. In order to determine ESBL type, we analyzed the *bla* gene.

The *bla* gene was detected by polymerase chain reaction (PCR) using nine primer pairs reported by Kojima et al. (3). Primers specific to the *bla*_{CTX-M-9} group gave positive gene amplification. The amplification product was sequenced by using ABI PRISM310 and the Big Dye Terminator V3.0 Cycle Sequencing kit (Applied Biosystems, Foster City, Calif., USA). A homology search in DDBJ using blast-n identified a complete match with the *Escherichia coli bla*_{CTX-M-14} gene (accession no. AF252622) (4).

As *bla*_{CTX-M-14} is generally plasmid-borne and transmitted horizontally, we investigated for the plasmids and their transferability (5). The plasmid DNA extracted from SE Y18809 according to the method by Kado and Liu (6) was electrophoresed in 1.0% agarose gel using 1 \times TAE buffer. As shown in Figure 1, two bands, ps1 and ps2, representing relatively large plasmids were detected. Of the two bands, only ps1 was hybridized by the PCR product obtained above, indicating that ps1 carried the drug resistance gene. The plasmid's transferability was examined by using a donor bacteria Y21134, an *E. coli* K12 DH10B (Invitrogen, Carlsbad, Calif., USA) transformed by the plasmid DNA (ps1) extracted from SE Y18809, and a recipient bacteria SE Y18808, a chicken meat-derived ampicillin-sensitive nalidixic acid-resistant

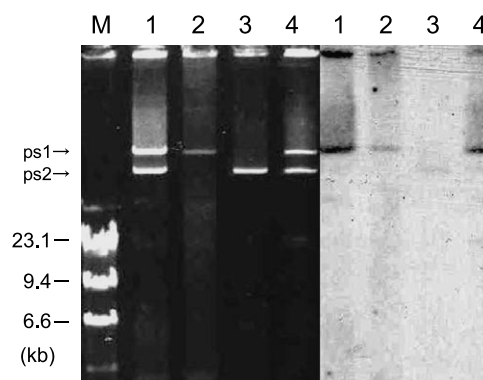


Fig. 1. Plasmid profile and Southern blot analysis of *Salmonella enterica* serovar Enteritidis (SE) Y18809 and its transformant. Lane 1, SE Y18809; Lane 2, *E. coli* Y21134 DH10B transformant of Y18809 (donor of transconjugation tests); Lane 3, SE Y18808 (recipient of transconjugation tests); Lane 4, SE Y21135 Y21134/Y18808 transconjugant. Lane M, a size marker of lambda DNA digested with *Hind*III.

strain. Transconjugants were selected on LB agar plates containing ampicillin (100 mg/L) and nalidixic acid (100 mg/L). The frequency of the drug resistance transmission was 10^{-1} . The plasmid profile on the gel and Southern hybridization experiments confirmed that the resulting transconjugant (coded as SE Y21135) harbored a plasmid corresponding to ps1 with the *bla*_{CTX-M-14} gene. The drug resistance pattern was almost identical to that of SE Y18809 (Table 1).

ESBL-producing SE has so far been reported in Spain in 2003 (type CTX-M-14), and in Hong Kong (type CTX-M-14), Poland (type CTX-M-3) and Japan (type CTX-M-14) in 2003 (5,7-9). All these were isolates from patients' blood or fecal specimens, but not from food. Our present finding suggests that the drug-resistant SE can be imported from abroad through food meat imports.

We compared ESBL-producing SE strain Hd63 (CTX-M-14 type) isolated from an outpatient's stool with our strain SE Y18809 (5). Their phage types were both 6a, and the pulsed-field gel electrophoresis (PFGE) patterns of *Bln*I- and *Xba*I-digested chromosomal DNA were indistinguishable from each other. The amino acid sequences in the QRDR region of *gyrA* responsible for nalidixic acid resistance, however, were not identical; i.e., Asp (GAC) at amino acid position 87 was replaced by Gly (GGC) in SE Y18809 while by Asn (CAC) in Hd63. This may indicate that these two resistant strains arose through different mutation events. It is possible that environments exist that favor the occurrence of drug-

resistant SE. Surveillance of drug-resistant pathogens in food or livestock should be further intensified.

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