Short Communication

Isolation of *Serratia fonticola* Producing FONA, a Minor Extended-Spectrum β -Lactamase (ESBL), from Imported Chicken Meat in Japan

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SUMMARY: Five novel strains of *Serratia fonticola* that produce FONA, a minor extended-spectrum beta-lactamase (ESBL), were isolated during routine surveillance of ESBL-producing *Enterobacteriaceae* in imported chicken meat in Japan in 2017 and 2018. These strains exhibited a clear ESBL phenotype in susceptibility tests carried out in the presence of clavulanic acid; however, all strains tested negative in a multiplex polymerase chain reaction assay used to detect TEM, SHV, and CTX-M β -lactamase genes. After identification of the bacterial species as *S. fonticola*, full length *bla*_{FONA} genes were amplified and the DNA sequences were determined. The *bla*_{FONA} genes from all 5 strains were different from those previously reported (*bla*_{FONA-1} to *bla*_{FONA-6}); they clustered close to one another but were distinct from previously reported *bla*_{FONA} genes in a phylogenetic analysis based on amino acid sequences.

Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* have emerged as one of the largest threats to patient safety in medical settings as they promote hydrolytic deactivation of both third- and fourth-generation cephalosporins, which are currently in use in hospitals worldwide (1). Moreover, genes encoding ESBLs typically reside on plasmids and, in some cases, on conjugative plasmids, facilitating rapid spread of resistance to commensal bacteria in the human gut. In fact, the most prevalent ESBL gene, *bla*_{CTX-M}, is believed to have translocated to a plasmid from a bacterial chromosome of a member of the genus *Kluyvera* (2). Among several minor ESBLs, SFO-1, which is encoded by a plasmid, exhibits high homology (96%) with FONA encoded by *Serratia fonticola* (3,4).

We obtained 5 novel *S. fonticola* isolates producing FONA as part of the surveillance for ESBL-producing *Enterobacteriaceae*. The isolates were obtained from a total of 176 samples of imported chicken meat at several quarantines in Japan in 2017 and 2018; 4 strains were detected in 86 chicken meat samples in 2017 and one was identified in 90 chicken meat samples in 2018. In this procedure, we first isolated ESBL-producing

Enterobacteriaceae by selection for cefotaxime (CTX) or ceftazidime (CAZ) resistance (each at 1 mg/L) on deoxycholate-hydrogen-sulfide-lactose agar. Resistant isolates were purified to determine the minimal inhibitory concentrations (MICs) of CTX and CAZ with or without clavulanic acid (CLA). The isolated strains with reduced resistance (1/8 or less of the MIC of CTX or CAZ) in the presence of CLA were further examined for the detection of the ESBL genes *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} by multiplex polymerase chain reaction (PCR) (5). PCR-negative isolates were further examined to identify constituent species using the BBL Crystal Enteric/Nonfermenter ID Kit (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) along with DNA sequencing of the 16S rRNA-encoding gene (6). Five strains were isolated and identified as *S. fonticola*. The results of susceptibility testing are shown in Table 1, together with additional information regarding chicken meat sources of the novel isolates. Although all 5 isolates were resistant to CTX and exhibited reduced resistance to CTX in the presence of CLA, they retained sufficient susceptibility to a variety of antibiotics, including CAZ. To confirm the presence of an ESBL gene, we performed PCR with the specific primers CTX-M/F (5'-TTTGCGATGTGCAGTACCAGTAA-3') and CTX-M/R (5'-CTCCGCTGCCGTTTATC-3') as reported previously (7). The resulting amplicons were sequenced. All the obtained sequences were amplification products of the internal regions of *bla*_{FONA}. The entire ORF of *bla*_{FONA} was amplified using the specific primers fonA-F1 (5'-ATGRTTARAAATA YAYTACGTMAAACCACCCTG-3') and fonA-R1

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Table 1. Antibiotic susceptibility of *S. fonticola* isolates from chicken meats¹⁾

Strain	Year	Original country	Accepting custom	AMP ²⁾	CAZ	CAZ/CLA ³⁾	CTX	CTX/CLA	FOX	FOX/BA ³⁾	IPM	MEPM	GEN	KAN	STR	AMK	TET	CIP
KT2480	2017	Brazil	Tokyo	128 ≤	≤ 1	0.5	128 ≤	0.5	8	8	0.5	≤ 0.25	≤ 0.25	0.5	0.5	0.5	4	≤ 0.25
KT2481	2017	Brazil	Naha	128 ≤	≤ 1	0.25	16	≤ 0.25	8	4	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	0.5	≤ 0.25	2	≤ 0.25
KT2482	2017	Brazil	Otaru	128 ≤	≤ 1	0.25	4	≤ 0.25	32	16	0.5	≤ 0.25	≤ 0.25	≤ 0.25	0.5	≤ 0.25	4	≤ 0.25
KT2483	2017	US	Kobe	128 ≤	≤ 1	0.5	64	1	≤ 4	4	1	≤ 0.25	≤ 0.25	0.5	2	0.5	4	≤ 0.25
KT2520	2018	Thailand	Osaka	128 ≤	≤ 1	0.5	8	≤ 0.25	16	8	0.5	≤ 0.25	≤ 0.25	0.5	1	0.5	4	≤ 0.25

¹⁾ Minimal inhibitory concentrations (MICs) were determined by agar dilution method and expressed in mg/L.²⁾ Abbreviations for the antibacterial agents used are as follows: ampicillin (AMP), cefoxitin (FOX), imipenem (IPM), meropenem (MEM), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), amikacin (AMK), tetracycline (TET), and ciprofloxacin (CIP).³⁾ CLA and BA (boric acid) were used to inhibit class A β -lactamase and AmpC cephalosporinase at the concentration of 10 mg/L and 300 mg/L, respectively.

('5-TCAAAGCCCTTCGGTRACAATTTTAGCAG CCGC-3'). The primers were designed based on the 6 *bla*_{FONA} sequences (*bla*_{FONA-1}–*bla*_{FONA-6}) deposited in the GenBank database. The DNA sequences of the 5 *fonA* genes were 888 bp in length with deduced amino acid sequences of 295 amino acids. A comparison of the DNA sequences of the 5 *bla*_{FONA} genes from these isolates with those of *bla*_{FONA-1} to *bla*_{FONA-6} revealed that they were highly homologous to one another and to the previous set of *bla*_{FONA} genes, although none were identical. CTX resistance was not transferred in solid mating studies ($< 10^{-7}$ per donor cell). We created a phylogenetic tree based on the deduced amino acid sequences of all known FONAs along with those of SFO-1 and CTX-M using CLUSTAL 2.1 Multiple Sequence Alignments and PhyML available at <https://www.genome.jp/tools-bin/clustalw>. The results of this analysis are shown in Fig. 1. Although the 5 newly identified FONAs clustered close to one another and with FONA-5, there was a distinct separation between this group and most of the previously reported FONAs except for FONA-5 and SFO-1. Comparison of the amino acid sequence of SFO-1 with those of the 5 new FONAs and with that of FONA-5 revealed 10 to 12 amino acid substitutions. A comparison of SFO-1 with FONA-1, -2, -3, -4, and -6 revealed 13 to 16 sites of amino acid sequence divergence. In contrast, there were only 2 or 3 amino acid substitutions when comparing the sequence of FONA-KT2480/FONA-5 group and 4 additional new FONAs to one another. These results suggest that the 5 new FONAs and FONA-5 were more similar to one another than any one of the groups was to SFO-1. FONAs from 4 of the novel strains (i.e., KT2481, KT2482, KT2483, and KT2520) fell within the same clade. Interestingly, these strains came from chicken meat samples from varying countries of export and from different years of examination (Table 1). Moreover, antibiotic susceptibility profiles and extent of resistance to CTX differed significantly among these isolates (Table 1). Therefore, it would be interesting to know which or whether all of the 5 strains isolated in this study originated from the same ancestor. It would also be interesting to have an idea that those *S. fonticola* have had the ability to stay in chicken and/or chicken meat and become a common contaminant in meat, although *S. fonticola* has been characterized as an environmental bacterium (8,9). These concepts remain speculative at this point and would require supporting evidence from multi-locus sequence typing analysis, notably due to the fact that their pulsed-field gel electrophoresis profiles differed from one another (data not shown).

S. fonticola has been recognized primarily as a bystander rather than as a pathogen in clinical settings and, as such, little attention has been paid to this bacterium despite reports documenting its isolation from wounds and the urinary tract (10–13). However, as *bla*_{SFO-1} currently identified in *Enterobacter cloacae* is believed to be derived from *bla*_{FONA} encoded in the *S. fonticola* chromosome (4), increasing prevalence and range of *S. fonticola* implies an enhanced risk of transfer of the ESBL gene *bla*_{FONA} to members of the family *Enterobacteriaceae* found in the human gut. As such, considerably more attention might be directed

Fig. 1. Phylogenetic relationships between deduced amino acid sequences of the FONAs and CTX-Ms. DNA sequences of 5 newly identified *bla*_{FONA} genes identified and described in this study were deposited in the GenBank database with the following accession numbers: KT2480 (LC522131), KT2481 (LC522132), KT2482 (LC522133), KT2483 (LC522134), and KT2520 (LC522135). The accession numbers of gene sequences encoding class A β -lactamases FONA-1 to FONA-6 are AJ251239 to AJ251244, respectively.

toward this bacterial species; at the very least, ongoing surveillance of antimicrobial-resistant bacteria found among contaminated meat should be continued.

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Conflict of interest None to declare.

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