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REVIEW

Phylogenetic Characterization of *Salmonella enterica* Serovar Typhimurium and its Monophasic Variant Isolated from Food Animals in Japan

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Abstract

Salmonella enterica subsp. *enterica* serovar Typhimurium and its monophasic variant (*Salmonella* 4,[5],12:i:-) are two of the most frequently isolated serovars in food animals worldwide. Especially, human and swine infections by *Salmonella* 4,[5],12:i:- have increased considerably in Western countries since the mid-1990s. Although bovine and swine salmonellosis by *Salmonella* 4,[5],12:i:- has been increasing in Japan, the genetic background of the serovar has been unclear. In our study, whole genome sequence (WGS)-based phylogenetic analysis was performed to reveal the characteristics of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolated in Japan. Nine distinctive clades were identified and clonal replacements were observed several times in the last four decades. Among the nine clades, clade 9, which is a multidrug-resistant and predominant clone after 2010, has similar characteristics to the European clone that has been a major threat to public and animal health worldwide in recent years. Furthermore, the two distinctive clades in clade 9 were identified by phylogenetic analysis. Each clade has diverged through microevolution mediated by the acquisition or deletion of mobile genetic elements such as plasmids, prophages, composite transposons, and integrative and conjugative elements. The present review focuses on WGS-based phylogenetic studies of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolated from food animals in Japan.

Discipline: Animal Science
Additional key words: clonal expansion, mobile genetic elements, whole genome sequencing

Introduction

Salmonella enterica subsp. *enterica* serovar Typhimurium is one of the most common gastroenteritis pathogens in humans and animals worldwide (Foley & Lynne 2008, Herikstad et al. 2002). Although this serovar has long been a major cause of salmonellosis in many countries, both human and animal infections by *Salmonella* 4,[5],12:i:-, which is a monophasic variant of *Salmonella* Typhimurium, have been increasing considerably since the mid-1990s in Japan (Ido et al. 2014, Kurosawa et al. 2012). A number of outbreaks caused by *Salmonella* 4,[5],12:i:- have been reported in many countries, and most of these cases have been linked to the consumption of contaminated pork and other sources, such as cattle and poultry (Arnott et al. 2018, Bone et al. 2010, Elnekave et al. 2018, Mossong et al. 2007, Tavechio et al. 2004, Trüpschuch et al. 2010, Yang et al. 2015). Sequence type 34, which is genotyed by multilocus sequence typing based on the nucleotide sequences of seven housekeeping genes, namely, *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrE*, *Salmonella* 4,[5],12:i:-, called European clone, is one of the best-studied clones due to its worldwide dissemination (Elnekave et al. 2018, Mather et al. 2018, Petrovska et al. 2016, Yang et al. 2015). This clone has been characterized

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by the possession of a composite transposon and integrative and conjugative elements (ICEs) that are responsible for antimicrobial resistance and heavy metal tolerance, respectively (Petrovska et al. 2016).

In Japan, *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- are two of the most common serovars of bovine salmonellosis. To better understand the epidemic clones among the isolates obtained from cattle in the 1990s and the 2000s, Tamamura et al. (2011) analyzed 545 *Salmonella* Typhimurium isolates obtained from cattle in Japan using macrorestriction analysis of XbaI-digested genomic DNA by pulsed-field gel electrophoresis (PFGE). Subsequently, PFGE clusters I and VII were identified as predominant clones in the 1990s and the 2000s, respectively. PFGE cluster I comprised definitive phage type DT104 and closely related isolates that had been a public health concern worldwide because of its multidrug resistance. PFGE cluster VII was also characterized by multidrug resistance because of the plasmids pYT1 (GenBank Accession No. AB576781) and pYT2 (GenBank Accession No. AB605179) carrying multiple antimicrobial resistance genes (Tamamura et al. 2013). In recent years, the incidence of bovine and swine salmonellosis caused by *Salmonella* 4,[5],12:i:- has been increasing in Japan. Ido et al. (2014) identified three different mutation patterns in the chromosomal region responsible for phase variation in *Salmonella* 4,[5],12:i:-. Although these findings suggest that there are several clones among *Salmonella* 4,[5],12:- isolates, the genetic background of *Salmonella* 4,[5],12:i:- isolated from food animals in Japan remains unclear.

The present review summarizes our molecular epidemiological studies on *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolated from food animals in Japan. Furthermore, the phylogenetic characteristics of the predominant clone of *Salmonella* 4,[5],12:i:- in recent years have been introduced as key factors in this review.

1. Phylogeny of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolated from food animals in Japan and the characteristics of major epidemic clones in the last four decades

Whole genome sequence-based phylogenetic analysis was performed using a total of 119 isolates: 52 *Salmonella* Typhimurium isolates obtained from cattle, swine, and birds in Japan between 1985 and 2014; 43 *Salmonella* 4,[5],12:i:- isolates obtained from cattle, swine, birds, meats, environmental water, and humans in Japan between 2000 and 2014; and 24 *Salmonella* 4,[5],12:i:- isolates obtained in Italy (Arai et al. 2018). A total of 6,205 single nucleotide polymorphisms (SNPs) were detected in the core genome region of the 119 isolates using *Salmonella* Typhimurium str. LT2 (Accession No. NC_003197.1) as a reference and concatenated to generate a phylogenetic tree with maximum likelihood. Nine clades were identified and discriminated against each other, and each clade had the following characteristics (Fig. 1). First, *Salmonella* Typhimurium isolates were mainly summarized in clades 1, 2, 3, 5, 6, and 7. Among these clades, clade 1 isolates correlated with PFGE cluster I in the study by Tamamura et al. (2011) and corresponded to definitive phage type 104 (DT104) and U302, which were threats to animal and public health worldwide in the 1990s (Helms et al. 2005, Tamamura et al. 2011). Typical clade 1 isolates showed resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline due to the presence of *Salmonella* genomic island 1, which contains an antimicrobial gene cluster (Boyd et al. 2001). Clade 7 correlated with PFGE cluster VII and comprised isolates obtained solely from cattle. These clade isolates typically possessed three prophages, namely, PsP3, P22, and P2-like, and were resistant to ampicillin, streptomycin, sulfonamides, tetracycline, and kanamycin. Most clade 7 isolates have a virulence-resistance plasmid, pYT1, carrying *spv* genes as virulence factors, and antimicrobial resistance gene clusters surrounded by two copies of IS1294 (Arai et al. 2018, Tamamura et al. 2013).

Salmonella 4,[5],12:i:- isolates were mainly grouped into clades 4, 8, and 9. Clade 4 exclusively comprised *Salmonella* 4,[5],12:i:- isolated from wild birds or environmental waters. Although most *Salmonella* 4,[5],12:i:- clade 8 and 9 isolates do not have *fljB*, clade 4 isolates have *fljA*, *fljB*, and *hin*, which are responsible for the phase variation of the H flagellar antigen with common amino acid substitutions, namely, A46T in FljA and R140L in Hin. Clade 8 isolates were obtained from various sources, including cattle, swine, humans, and environmental samples. Most clade 4 and 8 isolates were pansusceptible. Clade 9 consisted exclusively of sequence type 34 and was isolated from cattle, swine, and humans. The composite transposon carrying the antimicrobial resistance genes $bla_{\text{TEM-1B}}$, *strA*, *strB*, *sul2*, and *tet*(B), which is a specific element of the European clone (Garcia et al. 2016), was detectable in most clade 9 isolates. Thus, clade 9 isolates typically showed resistance to ampicillin, streptomycin, sulfonamides, and tetracycline. In addition, all clade 9 isolates had an integrative and conjugative element, ICEmST, which is a specific genomic island in the European clone (Petrovska et al. 2016). The ST34-specific ICE was first identified in *Salmonella* 4,[5],12:i:- and was designated

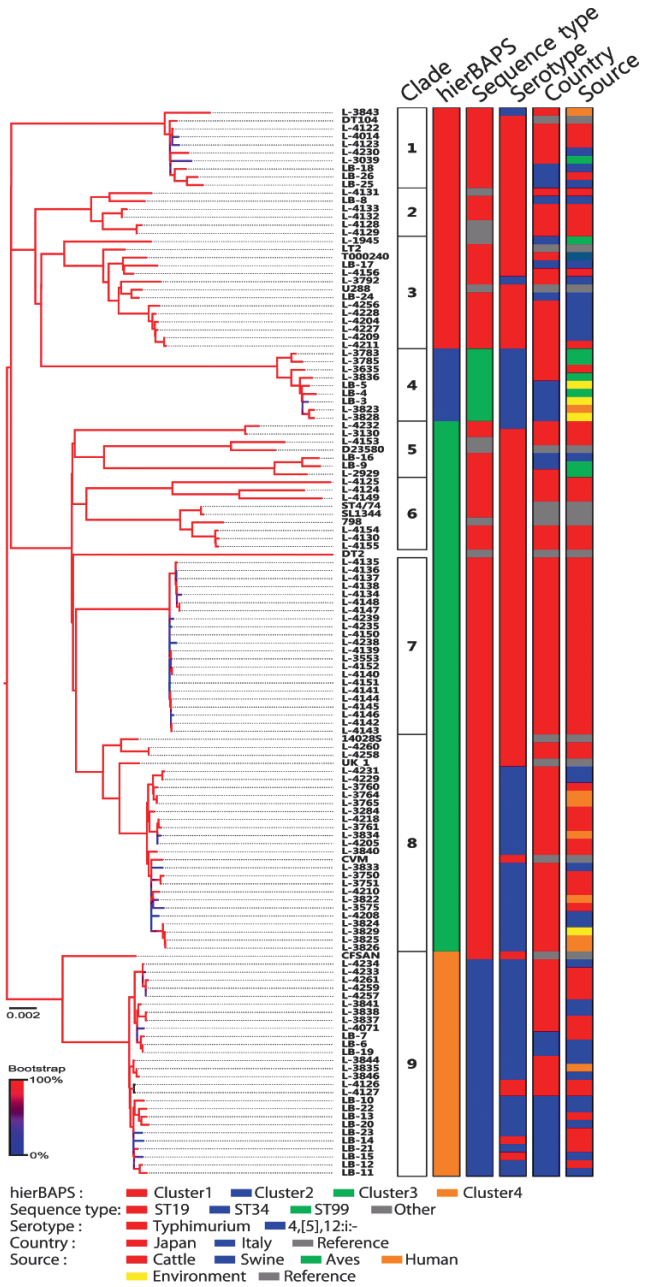

Fig. 1. Phylogeny of *Salmonella* Typhimurium and its monophasic variants in Japan and Italy isolated between 1984 and 2014 (Arai et al. 2018).
A phylogenetic tree was generated using maximum likelihood with 6,205 concatenated single nucleotide polymorphism in the core genome region among the 119 wild-type and 12 reference strains.

as *Salmonella* genomic island 3 (SGI-3) in 2016 (Petrovska et al. 2016). However, Moreno Switt et al. (2012) identified nine genomic islands in several *Salmonella* serovars and designated them SGI-2 to SGI-10. Therefore, we propose to redesignate the ST34-specific ICE as ICE in the monophasic variant of *Salmonella* Typhimurium (ICEmST) in the present study because the SGI number-based categorization of *Salmonella* ICEs is unclear (Arai et al. 2021).

To rapidly and simply identify which clade a tested isolate belonged to, we developed an SNP genotyping method that consisted of combinations of nine

allele-specific PCR (AS-PCR) to detect clade-specific SNPs. Each SNP genotype corresponds to the clade numbers. For example, the tested isolates positive for clade 1-specific SNP were designated as SNP genotype 1. We analyzed a total of 976 *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolates that were obtained from cattle and swine from 1976 to 2017 in Japan using SNP genotyping. The SNP genotypes of the 955 isolates were successfully determined. Among the 815 isolates from cattle, although SNP genotype 2 was the dominant type from 1985 to 1992, the epidemic genotype was clearly replaced by SNP genotype 2 to 1 in 1993 (Table 1). Subsequently, the dominant genotypes were changed to SNP 7 and 9 in 2004 and 2012, respectively. As for the temporal changes in cattle isolates, SNP genotypes 1 and 9 were the major types in the 1990s and the 2010s among 140 isolates from swine, respectively (Table 2). Furthermore, SNP genotype 3 was dominant

Table 1. SNP genotypes identified from 815 *S.* Typhimurium and *S. enterica* serovar 4,[5],12:i:- isolates from cattle between 1977 and 2017

Year of isolation	No. of isolates by SNP genotyping									Total no. of isolates
	1	2	3	4	5	6	7	8	9	
1977		1								1
1980						2				2
1981						1				1
1982						4				4
1983						1				1
1984			1			3				4
1985		6				4				10
1986	1	8	1							10
1987		9								9
1988		7								7
1989		7	2							9
1990	1	4								5
1991		13								13
1992	4	12			1					17
1993	27	3								30
1994	38	1	1							40
1995	22					2				24
1996	12					1				13
1997	21									21
1998	19				1			1	2	23
1999	28	1			1					30
2000	9		1			1	1	2		14
2001	13	1	1			1	1	1		18
2002	13	1				3	10			27
2003	18				3	3	12			36
2004	8					2	19	1		30
2005	3	2	2	1	1		46	2		57
2006		1	2			3	38			44
2007	11				3	2	46	1		63
2008	3				1	1	5	9	1	20
2009	2	1				2	1	4	4	14
2010	3						2	3	2	10
2011	1						1	1	2	5
2012	1	1					2	2	6	12
2013						3	1	6	18	28
2014		2	1				2		26	31
2015		2							58	60
2016	3	7					5		54	69
2017									3	3
Total	261	90	12	1	11	39	192	33	176	815

in swine isolates in 2011. SNP genotype 3 was a minor type (1.4%) in cattle isolates, whereas approximately 40% of swine isolates were discriminated into this genotype, suggesting that this genotype is more adapted to swine.

Clade 9 isolates were characterized by sequence type 34, composite transposons, and ICEmST, similar to the European clone, suggesting that this clade corresponds to the epidemic clone. European clones have rapidly disseminated in various countries, such as countries in Europe, North and South America, Oceania, and Asia (Arnott et al. 2018, Elnekave et al. 2018, Mather et al. 2018, Petrovska et al. 2016, Trüpschuch et al. 2010). The increased prevalence of clade 9 among food animals in Japan might be part of the pandemic of the European clone.

2. Characterization of clade 9-specific genetic element, ICEmST: integrative and conjugative element of the monophasic variant of *Salmonella* Typhimurium

ICEs are self-transmissible genomic elements that can excise from the host chromosome, form a circular intermediate, transfer by conjugation via a type IV secretion system, and integrate into the recipient chromosome (Bellanger et al. 2014, Johnson & Grossman 2015). ICEs are widely distributed in Gram-negative and Gram-positive bacteria (Delavat et al. 2017). Various cargo genes of ICEs have been reported, such as those responsible for antimicrobial resistance (Christie et al. 1987, Harada et al. 2010, Shoemaker et al. 1989, Whittle et al. 2002, Wozniak et al. 2009), biofilm formation (Carter et al. 2010), and metabolism of alternative carbon sources (Ravatn et al. 1998). A total

Table 2. SNP genotypes identified from 140 *S.* Typhimurium and *S. enterica* serovar 4,[5],12:i:- isolates from swine between 1976 and 2017

Year of isolation	No. of isolates by SNP genotyping									Total no. of isolates
	1	2	3	4	5	6	7	8	9	
1976		4								4
1977			1		1					2
1978		1								1
1982		2				4				6
1983		1								1
1988		4	5							9
1989		4								4
1991	1									1
1992			3							3
1993	1									1
1994	3									3
1995			3							3
1999										0
2000	2									2
2001	1		1					1		3
2002									1	1
2003								2	1	3
2004	1									1
2005	1							4		5
2006	2		3					1		6
2007	1		3					1		5
2008	1		3					1	2	7
2009			2		1				3	6
2010			4							4
2011	1		7	2						10
2012			5						2	7
2013			7					2	5	14
2014			2						4	6
2015			6						4	10
2016									11	11
2017									1	1
Total	15	16	55	2	2	4	0	12	34	140

of 86 open reading frames (ORFs) were identified in ICEmST, and 24 ORFs were predicted to be heavy metal resistance genes (Arai et al. 2019). Among the 24 ORFs, 17 formed a heavy metal homeostasis/resistance island called the copper homeostasis and silver resistance island (CHASRI). The CHASRI comprises three homeostasis/resistance systems: a plasmid-borne copper homeostasis system (*pco*) cluster (*pcoASCDRSE*), a *sil* heavy metal export system cluster (*silE* and *silP*), and a copper-sensing copper efflux system (*cus*) cluster (*cusABFCRS*) (Staehlin et al. 2016). The remaining seven ORFs were identified as the arsenic tolerance operon (*arsRDABC*).

As shown in Figure 2, ICEmST is an 81 kb genomic island carrying genes encoding a type IV secretion system and site-specific recombinase. Clade 9 *Salmonella* 4,[5],12:- str. L-3838 and L-3841 have ICEmST at the locus of the transfer RNA genes, namely, *pheV* and *pheR*, respectively. Although these observations suggest that ICEmST is an integrative and conjugative element, its function has been unclear. In our study, ICEmST of donor strain *Salmonella* 4,[5],12:i:- L-3841 (SNP genotype 9) was successfully transferred into the chromosome of the recipient strains of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- with SNP genotype 1 to 9 by the filter mating conjugation experiment (Arai et al. 2019). In addition, transconjugants were obtained from the other serovars: Heidelberg, Thompson, Hadar, Newport, and Cerro. The loci of two transfer RNA, namely, *pheV* and *pheR*, were identified as integrating sites. Furthermore, *attI*, indicating the generation of the circular form of ICEmST, was detected by PCR in the donor strain. After ICEmST integrated into the recipient chromosome, a direct repeat (*attL* and *attR*) was formed at the ends of ICEmST in the transconjugants (Fig. 3).

The minimum inhibitory concentration (MIC) of copper sulfate ($CuSO_4$) in the *Salmonella enterica* serovars used in our study ranged from 6 to 8 mM under aerobic conditions (Arai et al. 2019). Although the MIC of $CuSO_4$ in SNP genotype 9 strains L-3838 and L-3841 that have ICEmST was 6 mM, the strains of the other SNP genotypes and the other serovars showed MIC less than 1 mM under anaerobic conditions. By contrast, the MICs of the transconjugants to $CuSO_4$ under anaerobic conditions were four to six times higher than those of the wild-type strain. The MICs of $CuSO_4$ for L-3814ΔICEmST and LT2*pheR*ΔICEmST, which are ICEmST deletion mutants, were the same as those of LT2. Subsequently, although the MICs of L-3841Δpco and LT2*pheR*Δpco, which were deletion mutants of the pco gene cluster, were 1 mM, those of L-3841Δcus and LT2*pheR*Δcus were the same as those of the parental strains. The MICs of Na_2HAsO_4 for SNP genotype 9 strains under aerobic conditions were >64 μg/mL, although those of the other genotypes and serovars

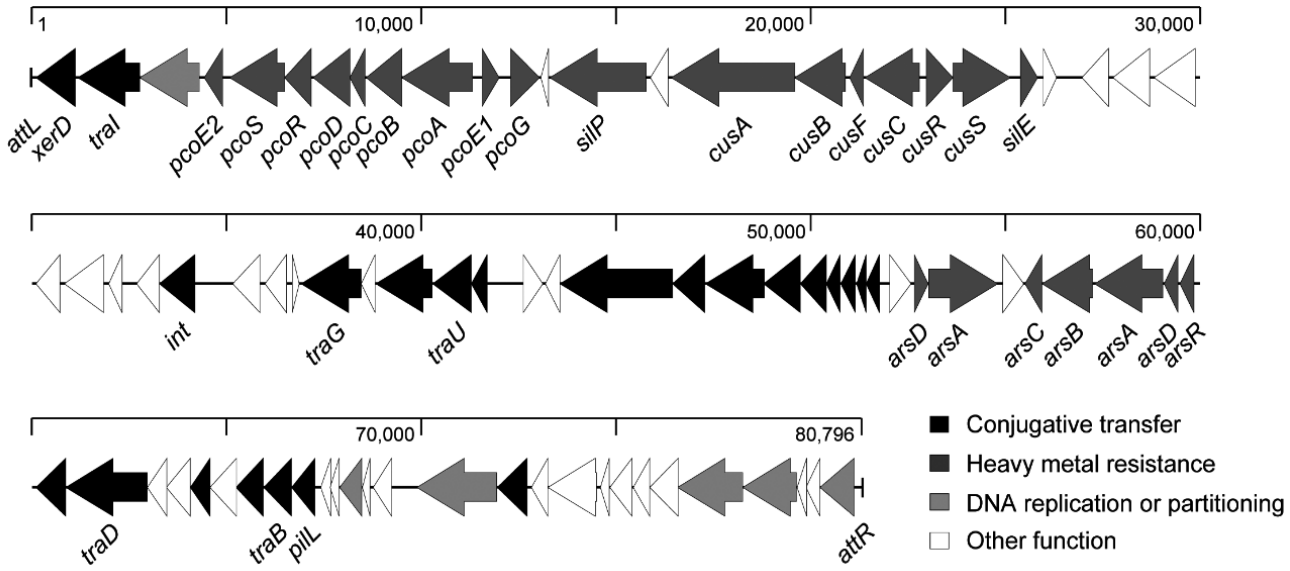

Fig. 2. Schematic view of full-length ICEmST of *Salmonella* 4,[5],12:i:- str. L-3841 (Arai et al. 2019)
The diagram shows the predicted classification of each gene that is represented by arrows according to the following scheme: black, genes for conjugative transfer; heavy gray, genes for heavy metal resistance; light gray, genes for DNA replication or partitioning; white, genes with other functions.

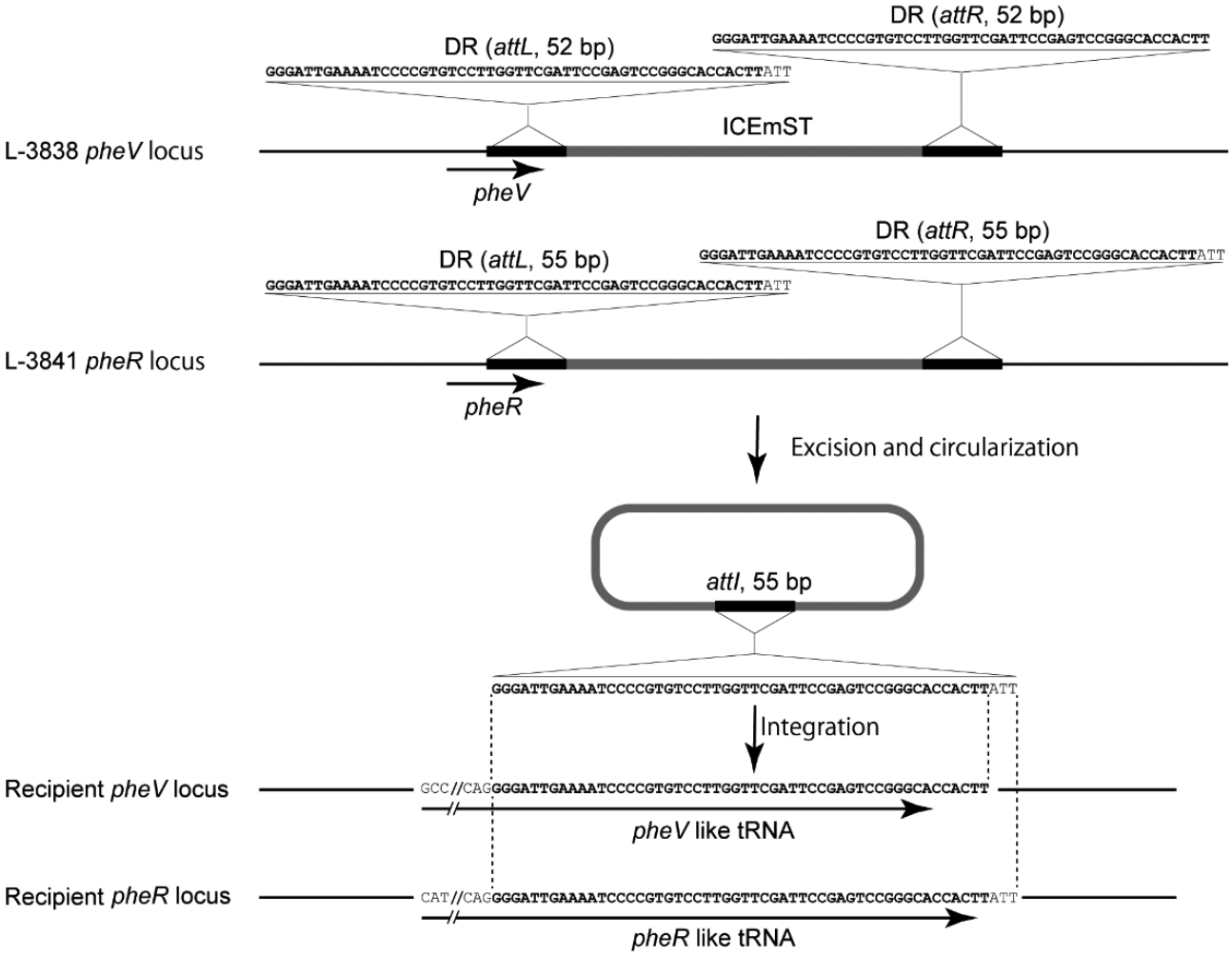

Fig. 3. The transfer flow of ICEmST (Arai et al. 2019)

Bold gray and black lines indicate ICEmST and a direct repeat (DR), respectively. Black file lines indicate bacterial chromosomes. ICEmST is located at the 3′ end of the tRNA gene *pheV* or *pheR* in donors. ICEmST is excised from the donors and transferred to the recipients. A circular form of ICEmST can be detected in both donors and recipients. ICEmST is integrated into the target site (*pheV* and *pheR*-like tRNA) of recipients and forms a DR at both ends. The DR sequence of ICEmST integrated into *pheV* and *pheR* was identical, but the DR located in *pheR* is 3 bp longer than that in *pheV*.

ranged from <0.25 to 4 μg/mL. The MICs of Na_2HAsO_4 of the transconjugants were at least 32-fold higher than those of the wild-type strain. These observations clearly suggest that ICEmST contributes to Cu tolerance under anaerobic conditions and As compounds under aerobic conditions. Copper tolerance is mainly defined by the *cus* gene cluster (Arai et al. 2019).

In the livestock industry, especially for pig breeding, heavy metals such as copper and zinc are used as micronutrients for growth promotion and suppression of enteric pathogens (Medardus et al. 2014). Aromatic organoarsenic compounds have been used in poultry farming (Jackson & Bertsch 2001). The copper concentration in swine feed samples in the United States was reported to range between 3.2 and 365.2 mg/kg (Medardus et al. 2014). In addition, it was reported that copper levels in swine fecal samples were higher, ranging from 71.2 to 2,397 mg/kg (Medardus et al. 2014). The use of high concentrations of heavy metals as feed additives might result in strong selection pressure for enteric bacteria. ICEmST contributes to copper tolerance under anaerobic conditions, suggesting that the acquisition of this genomic island might support S*almonella enterica* growth in an intestinal environment containing high concentrations of heavy metals.

3. Microevolution of clade 9 *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- shaped by the acquisition and loss of mobile genetic elements

Human and animal salmonellosis caused by

Salmonella 4,[5],12:i:- ST34 isolates, known as the European clone, have been reported worldwide. In Japan, *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- ST34/clade 9 isolates were first found in the 1990s and have become predominant in cattle and swine after the 2010s (Arai et al. 2018). Although ST34/clade 9 isolates were identified in the 1990s and the early 2000s, their number was relatively low during this period. Thus, it is unclear whether a single epidemic is responsible for the expansion of clade 9 strains among food animals in Japan.

A total of 214 *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolates that were isolated from cattle (n = 162), swine (n = 51), and humans (n = 1) were used for whole genome sequence-based phylogenetic analysis (Arai et al. 2021). The isolates were exclusively discriminated into SNP genotype 9 and were obtained from 16 of the 47 prefectures in Japan from 1998 to 2017. The 214 isolates were divided into two sublineages, designated as clade 9-1 and 9-2, using Bayesian phylogenetic analysis based on 911 SNPs in the core genome among 214 strains. Clade 9-1 consisted of 2 and 55 strains of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:-, respectively, which were isolated from 1998 to 2017. It was estimated that *Salmonella* 4,[5],12:i:- branched from *Salmonella* Typhimurium in approximately 1996. Among clade 9-1 strains, *Salmonella* 4,[5],12:i:- strains isolated in Japan from 2013 to 2017 formed a distinctive subclade 9-1a. All but one of the subclades, 9-1a, consisted of strains isolated from cattle. The Gifsy-1 prophage, which is one of the most common prophages in *Salmonella* Typhimurium, was not detected among all subclade 9-1a strains. Subsequently, clade 9-2 branched from the middle of clade 9-1, and it has become a dominant sublineage consisting of strains isolated from cattle and swine from 2012 to 2017. The emergence year of clade 9-2 was estimated to be 2000. In approximately 2002, three subclades, namely, 9-2a, 9-2b, and 9-2c, diverged. In other studies on the phylogenies of *Salmonella* Typhimurium (Petrovska et al. 2016) and *Escherichia coli* (Matsumura et al. 2016), the clusters were considered clonal expanding clades in case of a maximum node-to-tip distance of up to 70 SNPs. The number of SNPs among the epidemic subclades 9-2a, 9-2b, and 9-2c were 56, 47, and 67, respectively, suggesting that each subclade was associated with independent clonal expansion. Although all clade 9-2 strains lost the HP1 prophage, most subclade 9-2a and 9-2c members acquired the Col(pHAD28) and IncFIB/FIC plasmids, respectively. Five antimicrobial resistance (AMR) genes, namely, $bla_{\text{TEM1-B}}$, *strA*, *strB*, *sul2*, and *tet*(B), were carried on clade 9-specific transposon. AMR genes were detected in almost all 214 strains in this study. In addition to these five genes, *dfrA12*, *floR*, and *cmlA1* were detected in more than 20% of the 214 strains. These AMR genes are responsible for the resistance to trimethoprim and phenicols, and the strains' resistance to these antimicrobial agents, in addition to ASSuT (R-type ASSuTCTm), accounted for 18% of the total. In addition, the percentage of this R-type has increased from 2.6% before 2014 to 26% after 2015.

Salmonella Typhimurium can alternatively express two antigenically different flagellar proteins (H-antigens). *fljB* and *fliC* encode the phase 2 and 1 H-antigens, respectively. *fljA*, which is involved in the degradation of fliC mRNA, forms an operon with *fljB* (Yamamoto & Kutsukake 2006). The L-4126 strain, *Salmonella* Typhimurium clade 9-1, has both *fljAB* operon and a clade 9-specific composite transposon inserted between *hin* and *iroB* (Fig. 4). By contrast, the region between STM2760 and *hin* was deleted in 46 of the 57 strains in clade 9-1. Furthermore, L-4445, which belongs to *Salmonella* 4,[5],12:i:- clade 9-2, locked the region between STM2752 and *hin*. Expansion of the deleted region was observed in all members of clade 9-2. Two intact insertion sequences, 26 (IS*26*), were located at the end of the composite transposon. ISs are the simplest mobile genetic elements that induce various genomic rearrangements such as deletions, inversions, and duplications. On the basis of the genomic region around the composite transposon, the above stepwise deletions appeared to be caused by the intramolecular transposition of IS*26*.

Conclusion

In our study, nine clades were identified among *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- isolated from food animals in Japan. Clades 1, 7, and 9 mainly comprised multidrug-resistant isolates and were epidemic clones among cattle or swine over the last four decades. Clade 9 isolates have characteristics similar to those of European clones that are disseminated worldwide. Clade 9 isolates showed resistance to several antibiotics, as well as heavy metals, copper, and arsenic compounds. Because Cu is used as a micronutrient for growth promotion in swine, clade 9 isolates might have been retained under the selection pressure of both antibiotics and heavy metals. Clade 9 diverged into two subclades, namely, clade 9-1 and 9-2. These subclades were created by MGE-mediated microevolution steps, such as the acquisition of ICEmST, composite

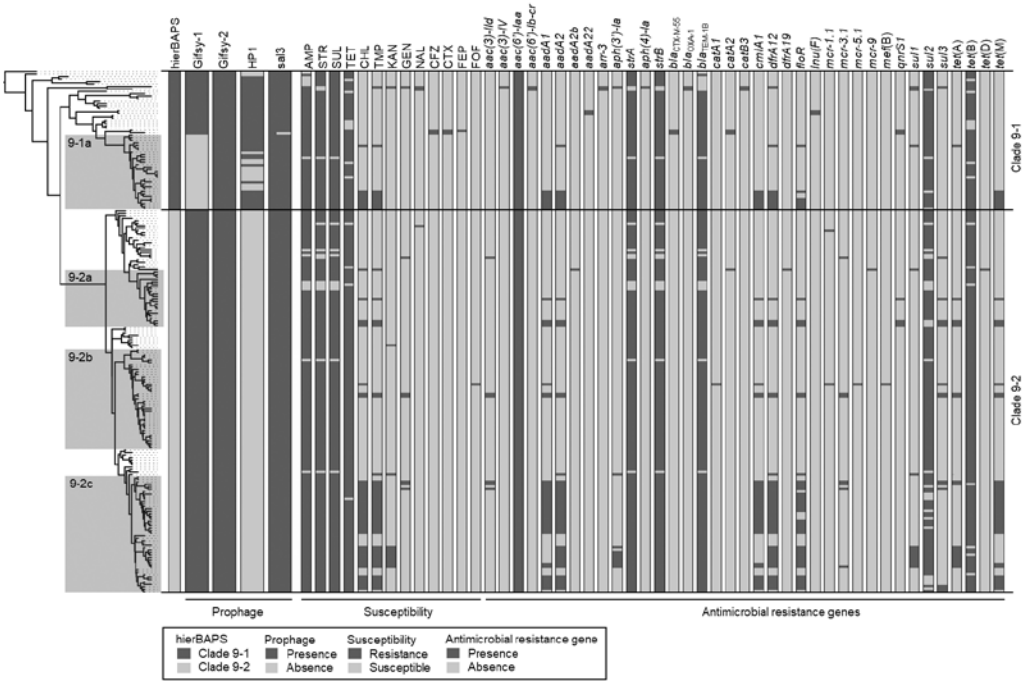

Fig. 4. Phylogeny of *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- ST34/SNP9 strains isolated in Japan between 1998 and 2017 (Arai et al. 2021)

A phylogenetic tree was generated using BEAST version 1.8.2 based on 911 concatenated SNPs in the core genomic sequences of 214 wild-type strains; hierBAPS cluster, susceptibility for each antimicrobial agents, and prevalence of prophages and antimicrobial resistance genes. The abbreviations are as follows: AMP, ampicillin; STR, streptomycin; SUL, sulfonamides; TET, tetracycline; CHL, chloramphenicol; TMP, trimethoprim; KAN, kanamycin; GEN, gentamicin; NAL, nalidixic acid; CFZ, cefazolin; CTX, cefotaxime; FEP, cefepime; FOF, fosfomycin.

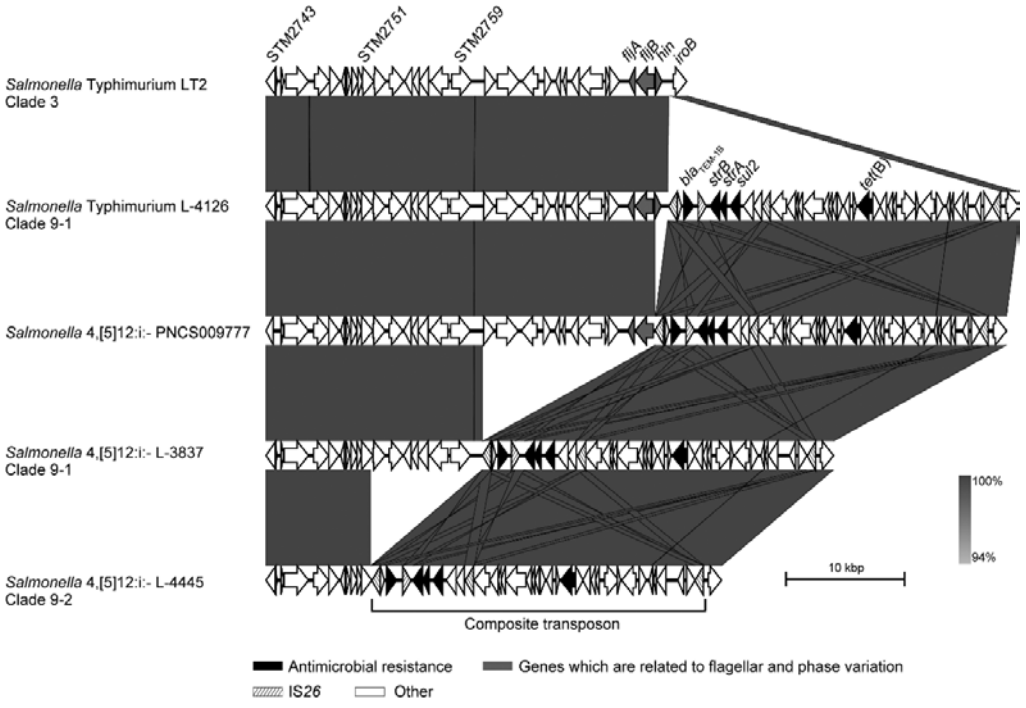

Fig. 5. Genomic structures of the flanking region of *fljB*, a phase 2 flagellar gene (Arai et al. 2021)

Comparison of genomic structures among non-clade 9 *Salmonella* Typhimurium, clade 9-1 *Salmonella* Typhimurium, clade 9-1 *Salmonella* 4,[5],12:i:-, and clade 9-2 *Salmonella* 4,[5],12:i:-

transposons, and plasmids, and deletion of prophages and the *fljAB* operon caused by IS*26*-mediated intramolecular transposition. Clade 9 isolates are still a major threat to salmonellosis among food animals in Japan, as well as in many other countries. Thus, we need a continuous and careful approach to reduce the risk of the expansion of this epidemic clone.

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