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Bacteriology

Genotypic and phenotypic characterization of *Salmonella enterica* subsp. *enterica* serovar Typhimurium monophasic variants isolated in Thailand and Japan

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ABSTRACT. Monophasic variants of *Salmonella enterica* serovar Typhimurium isolated in Thailand and Japan were characterized to elucidate the genetic basis of the monophasic phenotype, genetic relatedness, and antimicrobial resistance. A total of 20 *Salmonella* isolates agglutinated with anti-O4 and anti-H:i serum and not agglutinated with either anti-H:1 or anti-H:2 serum were identified as monophasic variants of *Salmonella* serovar Typhimurium because they harbored IS*200*, specific to this serovar, and lacked the *fljB* gene. An allele-specific PCR-based genotyping method that detects a clade-specific single nucleotide polymorphism indicated that seven swine isolates and one human isolate from Thailand were grouped into clade 1; five isolates from layer chicken houses and layer chicken feces from Japan were grouped into clade 8, together with two *Salmonella* serovar Typhimurium isolates from chicken houses in Japan; and five isolates from swine feces from Thailand and two isolates from layer chicken feces from Japan were grouped into clade 9. Multilocus sequencing typing demonstrated that sequence type (ST) 34 isolates were solely grouped into clade 9. Clade 1 and 8 isolates were assigned as ST19. Pulsed-field gel electrophoresis revealed multiple types within each of the clades. The presence of antimicrobial resistance genes and plasmid replicon type, of the clade 1 and 9 isolates were comparable to those reported for epidemic strains of monophasic variants. Our results suggest that monitoring monophasic variants of serovar Typhimurium is important for understanding of the spread of these variants in Thailand and Japan.

KEY WORDS: antimicrobial resistance, monophasic variant, *Salmonella* serovar Typhimurium, typing

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Salmonella enterica subsp. *enterica* serovar 4,[5],12:i:- (*Salmonella* serovar 4,[5],12:i:-), considered as a monophasic variant of *Salmonella* serovar Typhimurium that has lost the genes encoding the second flagellar antigens or the switching mechanisms of phase variation, has been increasingly isolated around the world [42], including in Thailand [1, 4, 21, 36] and Japan [22, 23, 25], since the mid-1990s. Whole or partial deletions or mutations in the *fljB* gene, encoding the phase 2 flagellin; the *fljA* gene, encoding a negative regulator of the phase 1 flagellin; and the *hin* gene, encoding an invertase regulating phase variation have been reported among monophasic variants in Thailand [21] and Japan [23].

The occurrence of *Salmonella* serovar 4,[5],12:i:- constitutes an animal husbandry and public hygiene concern because the pathogenicity of these strains in farm animals and humans is considered to be comparable to that of *Salmonella* serovar Typhimurium [13, 19]. Additionally, resistance to antimicrobials, including third-generation cephalosporins and plasmid- mediated quinolone resistance (PMQR) genes, have been reported in *Salmonella* serovar 4,[5],12:i:- strains [18]. It is generally believed that one of the reasons for the increased prevalence of antimicrobial-resistant strains in food-producing animals is the usage of antimicrobials in rearing practices [43]. Most *Salmonella* serovar 4,[5],12:i:- strains isolated in Thailand were multidrug resistant [21, 36], similar to European strains [20]. The antimicrobial resistance patterns of *Salmonella* serovar 4,[5],12:i:- in Japan range from pan-susceptible to multidrug resistant [23]. A recent study established an allele-specific PCR-based genotyping method that detects a clade-specific nucleotide polymorphism (SNP) and suggested that the increased detection of SNP genotype 9 (clade 9)

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among cattle and swine populations in Japan might be part of a pandemic of the European clone [2]. However, *Salmonella* serovar 4,[5],12:i:- strains isolated in Thailand have yet to be fully characterized.

In the present study, we carried out PCR mapping of the *fljBA* operon and its flanking region, including the *hin* gene, DNA-based typing, and antimicrobial susceptibility testing to elucidate the genetic characteristics and antimicrobial resistance patterns of swine fecal isolates and a human isolate from Thailand and compared these with isolates from laying chickens from Japan.

MATERIALS AND METHODS

Identification and serotyping of bacterial isolates

Twelve isolates were obtained from fecal samples of healthy pigs from four provinces in Thailand from 2012 to 2014. Eleven isolates were obtained from swab samples of chicken houses in a layer farm in prefecture 1 and from chicken feces from another layer farm in prefecture 2 in Japan from 2003 to 2014. A human isolate was recovered from a healthy carrier in Thailand. Identification of *Salmonella* was carried out based on colony morphology on selective media and biochemical properties. All isolates were serotyped using commercially available anti-*Salmonella* sera (Denka Seiken Co., Ltd., Tokyo, Japan). Isolates agglutinated with anti-O4 and anti-H:i serum and not agglutinated with either anti-H:1 or anti-H:2 serum were designated as "*Salmonella* serovar O4:i:-".

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the agar dilution method based on the guidelines provided by the Clinical Laboratory Standards Institute [12]. *Escherichia coli* ATCC 25922 was used as the quality control strain. MICs were interpreted using the resistance breakpoints defined in a previous study [24] as follows: ampicillin (AMP), 32 μg/m*l*; cefazolin (CEZ), 32 μg/m*l*; ceftiofur (CTF), 8 μg/m*l*; dihydrostreptomycin (DSM), 32 μg/m*l*; gentamicin (GEN), 16 μg/m*l*; kanamycin (KAN), 64 μg/m*l*; oxytetracycline (OTC), 16 μg/m*l*; chloramphenicol (CHL), 32 μg/m*l*; nalidixic acid (NAL), 32 mg/m*l*; enrofloxacin (ERFX), 2 μg/m*l*; and trimethoprim (TMP), 16 μg/m*l*. For sulfisoxazole (SUL), 512 μg/m*l* was adopted as a breakpoint according to CLSI document M100-S20 [11].

PCR

PCR targeting the *fljB-fljA* intergenic regions of *Salmonella* [15] was performed to confirm the presence of IS*200* between the *fljA* and *fljB* genes, which is specific to *Salmonella* serovar Typhimurium [5]. PCR mapping of the *fljBA* operon and its flanking region, including the *hin* gene, was performed to determine whether *Salmonella* serovar O4:i:- isolates maintain the *fljAB-hin* region [22]. Primers were designed to amplify the following regions [23]: up-*fljA*, *fljA* and its adjoining region; *fljA–fljB*, the boundary region of *fljA* and *fljB*; *fljB–hin*, the boundary region of *fljB* and *hin*; and *hin*-down, *hin* and its downstream region. All isolates were screened for plasmid-mediated quinolone resistance genes using multiplex (*qnrD* and *oqxAB*) or simplex (*qnrS, qnrA, qnrB, qnrC, qepA*, and *aac(6')-Ib-cr*) PCR [8, 10, 32, 37]. PCR detection of antimicrobial resistance genes in isolates interpreted as resistant to each of the antimicrobials was conducted using specific primers for: bla_{TEM} [7]; plasmid-mediated AmpC beta-lactamase genes [33]; *strA* and *strB* [27]; *aadA* [28]; *tet*(A), *tet*(B), and *tet*(G) [31]; *cat1*, *cat2*, *cat3*, and *floR* [29]; *sul1* [38], *sul2* [9], and *sul3* [17]; and *dhfrIb* and *dhfrX* [35].

Pulsed-field gel electrophoresis (PFGE)

The PFGE patterns of all isolates were analyzed as previously described [30]. Briefly, bacterial DNA prepared in an agar block was digested with 20 units of *Bln*I or *Xba*I and the DNA fragments were separated using a CHEF-DRII apparatus (Bio Rad Laboratories, Richmond, CA, U.S.A.) in 0.5 × Tris-borate-EDTA buffer supplemented with 50 μM thiourea at 14C and 6 V/cm. The switching times were increased from 1.0 to 50 sec during a total running time of 20.5 hr. Banding pattern analysis was performed with GelComparII version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium). Cluster analysis of the fingerprints obtained using a single enzyme was conducted by means of a similarity matrix calculation using the Dice coefficient followed by dendrogram construction using the unweighted pair group method with arithmetic mean (UPGMA) as the algorithm with optimization and tolerance set at 1% [3]. Isolates were assigned to genetically related clusters using the 80% strain similarity threshold [14] and distinguished numerically.

Multilocus sequencing typing (MLST)

The allele sequences of seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) were obtained using a 3130 genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.) and isolates were typed according to a publically accessible MLST database (http://mlst.ucc.ie/ mlst/dbs/Senterica).

SNP genotyping

A SNP genotyping system consisting of a combination of nine allele-specific PCRs [2] was applied to determine the clade to which the tested isolates belong.

Plasmid DNA analysis

Southern blot analysis was performed using the probe prepared from the PCR amplicon produced from primer pairs recognizing

Table 1. Origin, typing results, and DNA regions related to flagellar phase variation of the Salmonella isolates examined in this study

Isolate#	Area and country	Sorce	Isolation year	Serotype	PFGE type[a)]	MLST[b)]	Clade[c)]	Regions related to flagellar phase variation			
								up-*fljA*	*fljB-fljA*	*hin-fljB*	*hin*-down
D95	Province 3, Thailand	Swine feces	2012	O4: i: -	BlnI: 1-XbaI: 2	ST19	1	−	−	−	−
D97	Province 3, Thailand	Swine feces	2012	O4: i: -	BlnI: 1-XbaI: 2	ST19	1	−	−	−	−
E99	Province 4, Thailand	Swine feces	2013	O4: i: -	BlnI: 3-XbaI: 1	ST19	1	−	−	−	−
F35	Province 1, Thailand	Swine feces	2013	O4: i: -	BlnI: 3-XbaI: 1	ST19	1	−	−	−	−
PS57	Province 2, Thailand	Swine feces	2014	O4: i: -	BlnI: 9-XbaI: 3	ST34	9	−	−	−	−
PS60	Province 2, Thailand	Swine feces	2014	O4: i: -	BlnI: 9-XbaI: 3	ST34	9	−	−	−	−
PS63	Province 2, Thailand	Swine feces	2014	O4: i: -	BlnI: 9-XbaI: 3	ND	9	−	−	−	−
PS92.2	Province 2, Thailand	Swine feces	2014	O4: i: -	BlnI: 9-XbaI: 3	ST34	9	−	−	−	−
PS105	Province 2, Thailand	Swine feces	2014	O4: i: -	BlnI: 9-XbaI: 3	ST34	9	−	−	−	−
B81	Province 2, Thailand	Swine feces	2013	O4: i: -	BlnI: 4-XbaI: 1	ND	1	−	−	−	−
B86	Province 2, Thailand	Swine feces	2013	O4: i: -	BlnI: 3-XbaI: 1	ST19	1	−	−	−	−
B92	Province 2, Thailand	Swine feces	2013	O4: i: -	BlnI: 3-XbaI: 1	ND	1	−	−	−	−
B95	Province 2, Thailand	Human feces	2012	O4: i: -	BlnI: 2-XbaI: 2	ST19	1	−	−	−	−
S1743	Prefecture 1, Japan	Layer house environment	2001	O4: i: -	BlnI: 7-XbaI: 4	ND	8	−	−	−	+
S1821	Prefecture 1, Japan	Layer house environment	2004	O4: i: -	BlnI: 7-XbaI: 4	ND	8	−	−	−	+
S1910	Prefecture 1, Japan	Layer house environment	2004	Typhimurium	BlnI: 6-XbaI: 4	ST19	8	+	+	+	+
S1919	Prefecture 1, Japan	Layer house environment	2004	O4: i: -	BlnI: 7-XbaI: 4	ST19	8	−	−	−	+
S1935	Prefecture 1, Japan	Layer house environment	2004	Typhimurium	BlnI: 6-XbaI: 4	ST19	8	+	+	+	+
S1938	Prefecture 1, Japan	Layer house environment	2004	O4: i: -	BlnI: 7-XbaI: 4	ST19	8	−	−	−	+
S2617	Prefecture 1, Japan	Layer house environment	2012	Typhimurium	BlnI: 8-XbaI: 4	ST19	UT	+	+	+	+
S2618	Prefecture 1, Japan	Layer house environment	2012	Typhimurium	BlnI: 8-XbaI: 4	ST19	UT	+	+	+	+
S2689	Prefecture 2, Japan	Layer chicken feces	2014	O4: i: -	BlnI: 10-XbaI: 5	ST34	9	−	−	−	−
S2690	Prefecture 2, Japan	Layer chicken feces	2014	O4: i: -	BlnI: 5-XbaI: 4	ST19	8	−	−	−	+
S2691	Prefecture 2, Japan	Layer chicken feces	2014	O4: i: -	BlnI: 10-XbaI: 5	ND	9	−	−	−	−

a) Cluster analysis of the single enzyme fingerprints was conducted by means of a similarity matrix calculation using the Dice coefficient followed by a dendrogram constructed using the unweighted pair group method with arithmetic averages (UPGMA). Isolates were assigned to genetically related clusters using the 80% strain similarity threshold and distinguished numerically. b) Multilocus sequencing types. ND, not done. c) Results of an allele-specific PCR-based genotyping method detecting a clade-specific nucleotide polymorphism. UT, untypeable.

the IncA/C plasmid [6]. PCR-amplified fragments from one of the isolates positive for the IncA/C plasmid were labeled with digoxigenin using the DIG High Prime Labelling and Detection Starter Kit (Roche Diagnostics Corp., Indianapolis, IN, U.S.A.) and used as a specific probe. S1 nuclease-digested genomic DNA from selected isolates separated by PFGE according to the methods previously described [39] was transferred onto a Hybond-N+ membrane (Amersham Biosciences U.K. Ltd., Little Chalfont, U.K.). To determine the location of the bla_{TEM} gene, isolates harboring the bla_{TEM} gene were subjected to Southern blot analysis using a specific probe for the bla_{TEM} gene. PCR-amplified bla_{TEM} fragments from one of the AMP-resistant isolates were labeled with digoxigenin as described above.

RESULTS

Of the 24 isolates, 20 were serotyped as serovar O4:i:- and four isolates from environmental samples from a layer farm in Japan were serotyped as Typhimurium (Table 1). A 1,000-base pair amplicon of the *fljB-fljA* intergenic region, indicating the presence of *Salmonella* serovar Typhimurium-specific IS*200* [5], was detected in all 24 isolates. PCR mapping of the *fljBA* operon and its flanking region, including the *hin* gene, revealed that all isolates obtained in Thailand and two isolates from layer chicken feces from Japan lacked all these regions. Four isolates from environmental samples from a layer farm and an isolate from a chicken fecal sample from another farm in Japan were positive for only the *hin*-down region. The four serovar Typhimurium isolates were positive for all of the regions. Taken together, these results indicate that the absence of the *fljB* gene, which encodes the phase-2 flagellin, in serovar O4:i:- isolates in the present study is responsible for the monophasic phenotype.

*Bln*I- and *Xba*I-digested PFGE patterns were classified into 10 and five types, respectively (Fig. 1 and Table 1). Isolates assigned to *Xba*I-digested type 1 were classed into two different *Bln*I-digested types (3 and 4). Similarly, isolates belonging to *Xba*I-digested types 2 and 4 were separated into two (1 and 2) and four types (5 through 8), respectively, based on *Bln*I-digested patterns. The tested serotype O4:i:- and Typhimurium isolates were assigned to ST19 or ST34 by MLST analysis (Table 1). Both STs were found in isolates obtained in Thailand and Japan.

Twenty-two of the 24 isolates tested were assigned to clades 1, 8, or 9 using SNP genotyping (Table 1). All ST19 isolates obtained in Thailand were assigned to clade 1, whereas all those obtained in Japan, except for two, were assigned to clade 8. These two *Salmonella* serovar Typhimurium isolates were untypeable by SNP genotyping. Isolates considered to be genetically related based on PFGE analysis were grouped in a single SNP genotype.

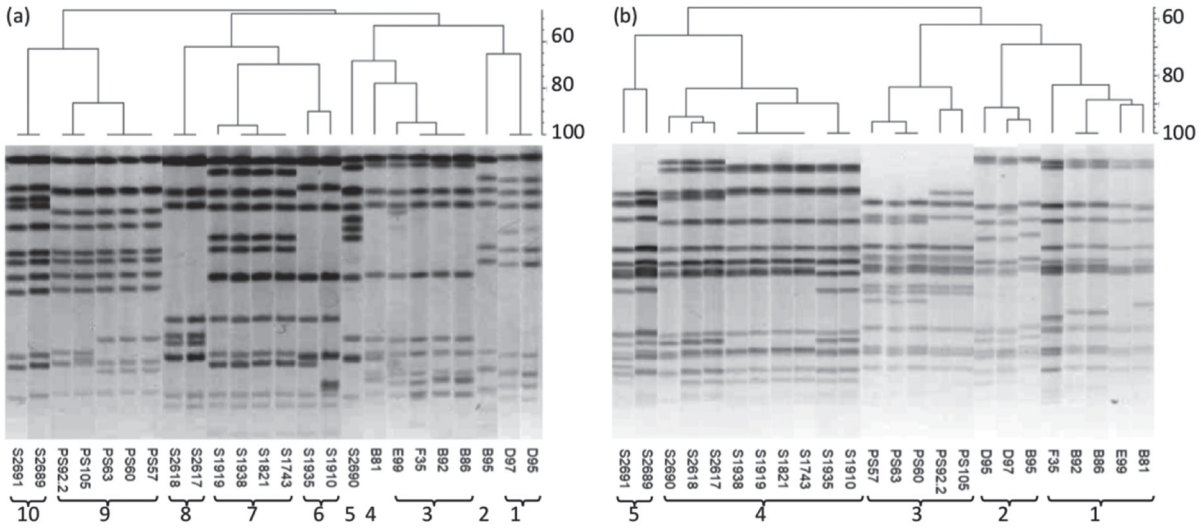

Fig. 1. PFGE patterns of *Bln*I- (a) and *Xba*I- (b) digested genomic DNA of the *Salmonella* isolates tested. Isolate number is indicated below each of the lanes (see Tables 1 and 2). The numbers below the parentheses refer to the PFGE types assigned to genetically related clusters based on the *Bln*I- (a) and *Xba*I- (b) digested patterns using the 80% strain similarity threshold.

All but one isolate from pig feces from Thailand were resistant to AMP, DSM, and OTC (Table 2). Resistance to GEN and NAL was detected in seven of the 12 isolates from swine feces, followed by SUL (five isolates), TMP (five isolates), CHL (four isolates), and KAN (one isolate). None of the isolates exhibited resistance to CEZ, CFT, or ERFX. In addition, none of the NAL-resistant isolates, including the human isolate, carried any PMQR genes. The NAL MIC values in these isolates ranged from 128 to 256 mg/*l*, suggesting that NAL resistance in these isolates is due to mutations in the quinolone-resistance determining regions of the *gyrA* and/or *parC* genes. All isolates from the environmental samples from the layer farm and layer chicken feces from Japan were resistant to DSM (Table 2). Two isolates from layer chicken feces were additionally resistant to OTC and SUL. Genes conferring resistance to beta-lactams, aminoglycosides, tetracyclines, phenicols, and sulfonamides were detected, whereas trimethoprim resistance genes were not found with the primer pairs used in this study (Table 2).

A recent study [2] revealed that a human monophasic mutant clade 1 isolate harbored the IncA/C plasmid conferring resistance to AMP, sulfonamides, GEN, streptomycin, tetracycline, and TMP. PCR-based replicon typing were performed and revealed that all eight clade 1 isolates from Thailand were positive for the primer pairs recognizing the IncA/C plasmid. Southern blot analysis using S1 nuclease-digested genomic DNA from these isolates separated by PFGE demonstrated that the probe was hybridized with plasmids, of which molecular weights ranged from approximately 120 to 180 kilobase pairs (Fig. 2). Furthermore, the bla_{TEM} gene was located on these plasmids (data not shown).

DISCUSSION

PCR mapping analysis revealed two types of amplification patterns, positive for only the *hin*-down and all negative, in the Japanese isolates. These patterns were previously detected in monophasic variants of *Salmonella* serovar Typhimurium obtained from animals and humans in Japan [23]. Ido *et al.* [23] also reported monophasic variants in which the entire *fljAB–hin* region was detected together with nucleotide mutations in the *fljA* and *hin* genes causing amino-acid substitutions in the FljA and Hin proteins, respectively. Huoy *et al.* [21] reported a distinct deletion pattern in the *fljAB* region in monophasic variants from various sources in Thailand.

The SNP genotyping results in the present study showing that all isolates assigned to ST34 by MLST were grouped into clade 9 are comparable to a recent report describing *Salmonella* serovar Typhimurium and O4:i:- isolates obtained from food-producing animals and humans in Japan and Italy [2]. Isolates belonging to clade 9 and ST34 in our study are characterized by the absence of the *fljBA* operon and its flanking region, including the *hin* gene, and the presence of *strA*, *strB*, *tet*(B), *sul2* (only in Japanese isolates), and bla_{TEM}. These characteristics are common to an endemic clone of a monophasic variant of serovar Typhimurium, the "European clone" [34], which is classified as ST34 and harbors a composite transposon insertion in the chromosome (containing the antimicrobial resistance genes described above) replacing the *fljAB–hin* region [2]. However, the PFGE type of clade 9 isolates obtained in Thailand was distinguished from that of Japanese isolates. Similar observations that monophasic variants designated as a single MLST type represented considerable PFGE diversity were previously reported [40]. In our study, several clade 1 isolates from Thailand were assigned to ST19 and harbored bla_{TEM}, which is assumed to be on an IncA/C plasmid, as well as *aadA*, *tet*(A), *sul1*, *sul2*, and *sul3*. These characteristics were also found in a human isolate from Japan reported by Arai *et al.* [2], which was assumed to be the "Spanish clone", the other epidemic clone [26, 40]. However, a detailed investigation, including analysis of

Table 2. Antimicrobial resistance phenotype and distribution of antimicrobial resistance genes

Isolate#	Antimicrobial resistance phenotype[a)]	Antimicrobial resistances gene tested[b)]																
		bla$_{TEM}$	AmpC[c)]	*strA*	*strB*	*aadA*	*tet*(A)	*tet*(B)	*tet*(G)	*cat1*	*cat2*	*cat3*	*floR*	PMQR[d)]	*sul1*	*sul2*	*sul3*	*dhfr1b*	*dhfrX*
D95	AMP-DSM-GEN-OTC-NAL	+	−	−	−	+	+	−	−					−					
D97	AMP-DSM-GEN-OTC-NAL	+	−	−	−	+	+	−	−					−					
E99	AMP-DSM-GEN-OTC-NAL-SUL-TMP	+	−	−	−	+	+	−	−					−	+	+	−	−	−
F35	AMP-DSM-GEN-KAN-OTC-CHL-NAL-SUL-TMP	+	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	−
PS57	AMP-DSM-OTC	+	−	+	+	−	−	+	−					−					
PS60	AMP-DSM-OTC	+	−	+	+	−	−	+	−					−					
PS63	AMP-DSM-OTC	+	−	+	+	−	−	+	−					−					
PS92.2	DSM-OTC	−	−	−	−	−	−	+	−					−					
PS105	AMP-DSM-OTC	+	−	+	+	−	−	+	−					−					
B81	AMP-DSM-GEN-OTC-CHL-NAL-SUL-TMP	+	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	−
B86	AMP-DSM-GEN-OTC-CHL-NAL-SUL-TMP	+	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	−
B92	AMP-DSM-GEN-OTC-CHL-NAL-SUL-TMP	+	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	−
B95	AMP-DSM-GEN-OTC-CHL-NAL-SUL-TMP	+	−	+	+	+	+	−	−	−	−	−	+	−	+	+	+	−	−
S1743	DSM			−	−	−								−					
S1821	DSM			−	−	−								−					
S1910	DSM			−	−	−								−					
S1919	DSM			−	−	−								−					
S1935	DSM			−	−	−								−					
S1938	DSM			−	−	−								−					
S2617	DSM			−	−	−								−					
S2618	DSM			−	−	−								−					
S2689	DSM-OTC-SUL			+	+	−	−	+						−	−	+	−		
S2690	DSM			−	−	−								−					
S2691	DSM-OTC-SUL			+	+	−	−	+						−	−	+	−		

a) AMP, ampicillin; DSM, dihydrostreptomycin; GEN, gentamicin; KAN, kanamycin; OTC, oxytetracycline; CHL, chloramphenicol; NAL, nalidixic aid; SUL, sulfisoxazole; TMP, trimethoprim. b) +, positive; -, negtive; blank, not done. c) AmpC, plasmid-mediated AmpC beta-lactamase genes. d) PMQR, plasmid-mediated quinolone resistance genes.

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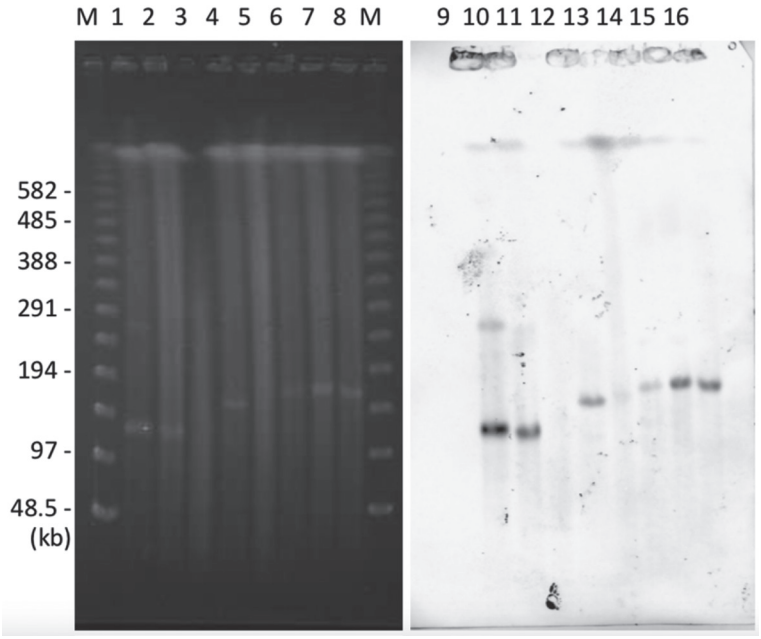

Fig. 2. PFGE analysis of S1 nuclease-digested genomic DNA of clade 1 *Salmonella* isolates and Southern blot hybridization with a probe prepared from the PCR amplicon from primer pairs recognizing the IncA/C plasmid. Lanes 1 to 8, PFGE of S1 nuclease-digested genomic DNA. Lanes: 1, D95; 2, D97; 3, E99; 4, F35; 5, B81; 6, B86; 7, B92; 8, B95; M, lambda ladder. Lanes 9 to 16, Southern blot hybridization analysis. The isolates in lanes 9 to 16 are the same as those in lanes 1 to 8. DNA of isolates E99 was unfortunately degraded.

integrons and transposons, and conjugation experiments are still required to determine whether the clade 9 and 1 isolates in our study are part of the European and Spanish clones, respectively, or not.

Resistance phenotypes varied among the isolates in our study. Swine isolates showed resistance to multiple drugs similar to that reported in antimicrobial resistant *Escherichia coli* isolates obtained from pigs in north-eastern Thailand [41]. The human clade 1 isolate in our study harbored the *strA*, *strB*, and *floR* genes in addition to resistance genes present in the clade 1 swine isolates. In contrast, isolates obtained from layer chickens or environmental samples from layer chicken houses were resistant to only a few antimicrobials. A possible explanation for this is that antimicrobials, including feed additives, are not usually used during egg production cycles. Although we did not analyze virulence determinants, the selective advantage of *Salmonella* serovar 4,[5],12:i:- strains prevalent in certain environments may be conferred not only by antimicrobial resistance, but also by virulence factors [16].

The clade 8 isolates in our study were obtained solely from Japan and were susceptible to all the antimicrobials tested, except for DSM. *Salmonella* serovar Typhimurium isolates were grouped into clade 8 together with the monophasic variant isolates; all clade 8 isolates were classified solely as ST19. Moreover, the *Xba*I-digested PFGE patterns of the serovar Typhimurium isolates were closely related to those of the clade 8 monophasic isolates. Our findings support the hypothesis that the clade 8 monophasic variants may have originated from serovar Typhimurium strains in this clade. Both monophasic variants and serovar Typhimurium isolates were grouped into clade 8 in a recent Japanese study [2]. However, most clade 8 isolates in the same study [2] were pan-susceptible.

DNA-based typing methods identified the presence of multiple monophasic variant strains in both countries, although only a limited number of isolates were analyzed in the present study, suggesting that monophasic variants of serovar Typhimurium have widely spread in these countries. Because isolates in this study were obtained from different animals in different years, this may influences the results as indispensable factors. Continuous monitoring of monophasic variants of serovar Typhimurium is imperative for gaining a clearer picture of the dissemination of these variants in Thailand and Japan, and understanding the global spread of these variants.

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