NOTE *Bacteriology*

Characterization of *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar 4,[5],12:i:- isolates from pigs presenting with diarrhea in Korea

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ABSTRACT: Between 2011 and 2012, a total of 896 pig fecal samples were collected from nine provinces in Korea, and 50 *salmonella enterica* susp. *enterica* serovar Typhimurium (*S*. Typhimurium) was isolated. The characteristics of the 50 strains were analyzed, and 4 strains were identified as *Salmonella enterica* subsp. *enterica* serovar 4,[5],12:i:-. *Salmonella* 4,[5],12:i:- could not be distinguished from *S*. Typhimurium through phage typing, antimicrobial resistance testing or multiple-locus variable-number tandem repeat analysis (MLVA). However, among the four *Salmonella* 4,[5],12:i:- strains, one (KVCC-BA1400078) was identified as a *Salmonella* 4,[5],12:i:- clone isolated from humans in the United States, and another (KVCC-BA1400080) was identified as DT193, which has been primarily isolated from humans and animals in European countries. The presence of *Salmonella* 4,[5],12:i:- in Korea poses a significant threat of horizontal transfer between pigs and humans.

KEY WORDS: Korea, *Salmonella* 4,[5],12:i:-, *S*. Typhimurium, swine

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Salmonella spp. are important zoonotic pathogens commonly found in farmed animals. In pigs, salmonellosis is an infectious digestive disease that presents with acute or chronic symptoms. In surveys of *Salmonella* in pigs, *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S*. Typhimurium) and *S.* Derby are commonly isolated worldwide, whereas the host-adapted *S.* Choleraesuis is now rare in Europe and Australia but still frequently found in North America and Asia. In Korea, *S*. Typhimurium is the main cause of digestive disease in pigs [4, 25].

Recently, there have been many reports of *S*. Typhimurium variants isolated from humans and warm-blooded animals [13]. Among these variants, *Salmonella enterica* subsp. *enterica* serovar 4,[5],12:i:- (*Salmonella* 4,[5],12:i:-) lacks expression of the second flagellar phase of *S*. Typhimurium, which has the antigenic formula 4,[5],12:i:1,2 [5]. *Salmonella* 4,[5],12:i:- has the O4 antigen, B serogroup and i antigen, but lacks the 1,2 antigen. Flagellar phase variation is induced by inversion of the genetic region called the H segment, which contains the *hin* gene encoding DNA invertase and a promoter for the *fljB* gene. The *fljB* gene constitutes an operon with the *fljA* gene [5]. Therefore, *S.* Typhimurium variants result from alterations in the expression of *fljA*, *fljB* and *hin* genes [16, 19]. The European Food Safety Authority has suggested a polymerase chain reaction (PCR) protocol supporting traditional slide agglutination serotyping to clearly differentiate *Salmonella* 4,[5],12:i:- from *S.* Typhimurium [5]. Since *Salmonella* 4,[5],12:i:- was first isolated in Spain [9], the serovar has been considered to be the predominant agent of food poisoning in Europe and the United States over the last 10 years [16]. In Korea, the first outbreak of food poisoning caused by *Salmonella* 4,[5],12:i:- was reported in 2001, and *Salmonella* 4,[5],12:i:- was isolated from poultry in 2009–2011 [21, 22]. However, there are no reports of *Salmonella* 4,[5],12:i:- isolates from cattle or pigs in Korea.

Epidemiological studies of *S.* Typhimurium have been carried out using classical methods, such as phage typing and antimicrobial resistance testing and molecular genetic methods, such as multiple-locus variable-number tandem repeat analysis (MLVA) and pulsed-field gel electrophoresis (PFGE) [2, 6, 14, 18, 23, 28]. Among these tools, MLVA is based on multiplex PCR amplification of VNTR (variable-number tandem repeat) loci followed by sizing of the products using capillary electrophoresis. The method is highly discriminatory, and, unlike PFGE, it is easy to perform, robust and rapid [12, 13, 20].

In this study, we examined the prevalence of *S.* Typhimurium in pigs presenting with diarrhea in South Korea, and we isolated *Salmonella* 4,[5],12:i- from farmed pigs for the first time. In addition, phage typing, antimicrobial resistance tests, flagellar gene distribution analyses and MLVAs were used to compare the characteristics of *S.* Typhimurium and *Salmonella* 4,[5],12:i:-.

Porcine fecal samples were collected from livestock presenting with digestive diseases, such as diarrhea and enteritis, by Choong-Ang Vaccine Laboratories. A total of 896 fecal samples were obtained from nine provinces (Gyeonggi, Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam and Jeju) throughout

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Table 1. List of the oligonucleotide primers used for PCR of *Salmonella*

Gene	Function	Nucleotide sequence (5′-3′)	Size of amplicon (bp)	Ref.
STM 0292	Putative RHS-family protein	ATGCGGGTATGACAAACCCT TTAGCCCCATTTGGACCTTT	94	[1]
STM 2235	Putative phage protein	CAGACCAGGTAAGTTTCTGG CGCATATTTGGTGCAGAAAT	196	[1]
STM 4493	Putative cytoplasmic protein	TTTACCTCAATGGCGGAACC CCCAAAAGCTGGGTTAGCAA	303	[1]
STM 1053–1997	-	CCATTTTTATACTGCCAGTCGCC CAGCGAAATACTGATGGCGG	614	[27]
STM 2740	Integrase, phage family	AATGTGGAGATCGCTGGCGCG AGTTCGCCGCCGAACCCC	980	[27]
STM 2757	Putative cytoplasmic protein	ATGATGATGGCGTAATGGCGC AAAACGTTCCGGTGCGGCG	717	[27]
STM 2773	Glucosyltransferase homolog	TTCGATTCGGAAGCGGGTTATCGCCG CTCGCGAAGCGCGCG	858	[27]
fljA	Repressor of phase 1 Flagellin gene	TTCATTAGGTCCCCTCCGG ATTCAGCCCCGTGAATTCGGG	642	[27]
fljB	Phase 2 flagellin structural protein	TTTACCGTCTACGCCACCC GGTACTACACTGGATGTATCGGG	561	[27]
hin	H inversion: regulation of flagellar gene expression	TGGCTACTATTGGGTATATTCGGG CTCGCGAAGCGCGCG	570	[27]

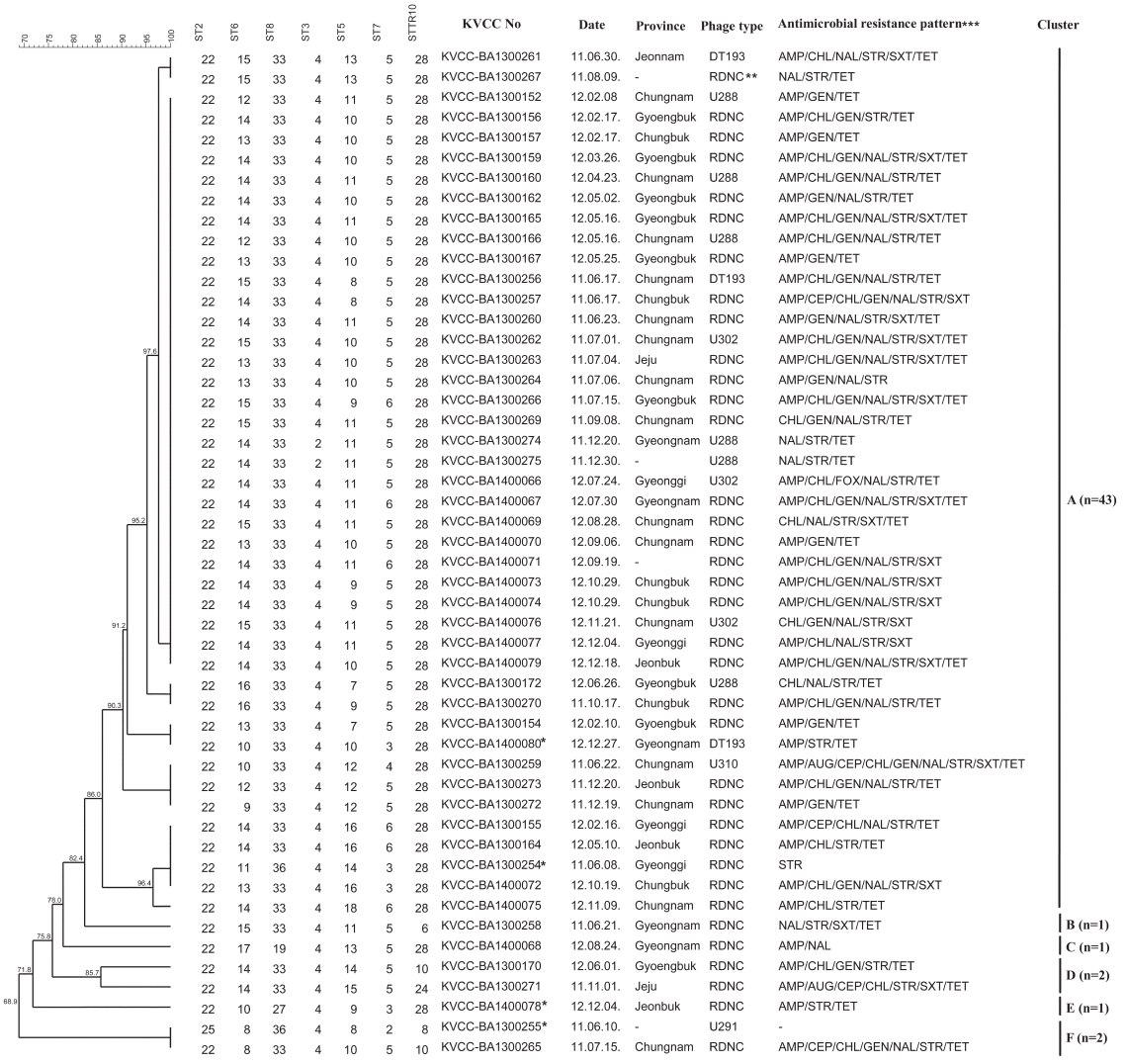
the Republic of Korea from 2011 to 2012. All samples were collected under aseptic conditions, mixed with 45 m*l* of buffered peptone water and incubated for 20 hr at 37°C. After incubation, 0.1 m*l* of each sample was inoculated into 10 m*l* of Rappaport Vassiliadis (RV) R10 broth (Merck, Darmstadt, Germany) and then incubated for 24 hr at 42°C. One loop of RV culture was streaked onto the surface of both xylose lysine deoxycholate agar (Difco, Becton, Dickinson, and Co., Sparks, MD, U.S.A.) and Salmonella Shigella agar (Difco, Becton, Dickinson, and Co.) plates, and then, suspected colonies were serotyped using *Salmonella* antisera (Denka Seiken, Tokyo, Japan) according to the method of Ewing [11]. Phage types were determined using *S.* Typhimurium phages obtained from Public Health England public health laboratories (Colindale, London, U.K.). Multiplex PCR was performed to detect the *S.* Typhimurium-related genes STM0292, STM2235 and STM4493 (Table 1) [1]. Isolates of *S.* Typhimurium were deposited in the Korea Veterinary Culture Collection (KVCC), where they were stored until further use.

Minimal inhibitory concentrations were determined by broth microdilution using the Sensititre microdilution panel by Thermofisher Inc. (formerly TREK Diagnostic Systems; Cleveland, OH, U.S.A.). The tested antimicrobial agents were ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, nalidixic acid, neomycin, streptomycin, tetracycline and trimethoprim-sulfamethoxazole. Susceptibility was determined in accordance with the guidelines of the Clinical and Laboratory Standards Institute [8]. *Escherichia coli* ATCC 25922 was used as a control strain.

The expression of flagellar genes (STM1053-1997, STM2740, STM2757, STM2773, *fljA*, *fljB* and *hin*) was assessed by PCR (Table 1) as described previously [27]. DNA fragments were separated on a 1.5% agarose gel. Fragments of the appropriate size were extracted from the gel and purified using a Gel Extraction Kit (Qiagen Inc., Valencia, CA, U.S.A.), followed by sequencing. A database search was performed using the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

MLVA was performed following the standardized procedure established by pulseNet [3]. The profiles of the isolates were compared using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) with the categorical coefficient and unweighted-pair group method with average linkages.

Salmonella spp. were tried to be isolated from nine provinces between 2011 and 2012. Fifty-nine *Salmonella* spp. out of 896 diarrheic pigs samples were isolated. Out of these isolates, 50 were identified as *S.* Typhimurium. In total, *S.* Typhimurium was isolated from 7.19% (21/292) of the pigs examined in 2011 and 4.8% (29/604) of the pigs examined in 2012. The total numbers of *S.* Typhimurium isolates from each region during the study period were as follows: Chungnam (n=15), Gyeongbuk (n=9), Chungbuk (n=6), Gyeongnam (n=5), Gyeonggi (n=4), Jeonbuk (n=4), Jeju (n=2), Jeonnam (n=1), Gangwon (n=0) and unknown (n=4). The seasonal frequencies of *S.* Typhimurium incidences on pig farms in 2011–2012 were 0.0–10.0% from January to March, 2.0–20.0% from April to June, 6.0–14.0% from July to September and 6.0–16.0% from October to December. The phage typing results for the *S.* Typhimurium isolates in this study were as follows: U288, 6 strains; U302, 3 strains; DT193, 3 strains; U310, 1 strain; U291, 1 strain; and reacted, but did not conform (RDNC), 36 strains. Among the *S.* Typhimurium isolates, four strains (KVCC-BA1300254, KVCC-BA1300255, KVCC-BA1400078 and KVCC-



ST2	ST6	ST8	ST3	ST5	ST7	STTR10	KVCC No	Date	Province	Phage type	Antimicrobial resistance pattern***	Cluster
22	15	33	4	13	5	28	KVCC-BA1300261	11.06.30.	Jeonnam	DT193	AMP/CHL/NAL/STR/SXT/TET	
22	15	33	4	13	5	28	KVCC-BA1300267	11.08.09.	-	RDNC**	NAL/STR/TET	
22	12	33	4	11	5	28	KVCC-BA1300152	12.02.08	Chungnam	U288	AMP/GEN/TET	
22	14	33	4	10	5	28	KVCC-BA1300156	12.02.17.	Gyoengbuk	RDNC	AMP/CHL/GEN/STR/TET	
22	13	33	4	10	5	28	KVCC-BA1300157	12.02.17.	Chungbuk	RDNC	AMP/GEN/TET	
22	14	33	4	10	5	28	KVCC-BA1300159	12.03.26.	Gyoengbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	14	33	4	11	5	28	KVCC-BA1300160	12.04.23.	Chungnam	U288	AMP/CHL/GEN/NAL/STR/TET	
22	14	33	4	10	5	28	KVCC-BA1300162	12.05.02.	Gyeongbuk	RDNC	AMP/GEN/NAL/STR/TET	
22	14	33	4	11	5	28	KVCC-BA1300165	12.05.16.	Gyeongbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	12	33	4	10	5	28	KVCC-BA1300166	12.05.16.	Chungnam	U288	AMP/CHL/GEN/NAL/STR/TET	
22	13	33	4	10	5	28	KVCC-BA1300167	12.05.25.	Gyeongbuk	RDNC	AMP/GEN/TET	
22	15	33	4	8	5	28	KVCC-BA1300256	11.06.17.	Chungnam	DT193	AMP/CHL/GEN/NAL/STR/TET	
22	14	33	4	8	5	28	KVCC-BA1300257	11.06.17.	Chungbuk	RDNC	AMP/CEP/CHL/GEN/NAL/STR/SXT	
22	14	33	4	11	5	28	KVCC-BA1300260	11.06.23.	Chungnam	RDNC	AMP/GEN/NAL/STR/SXT/TET	
22	15	33	4	10	5	28	KVCC-BA1300262	11.07.01.	Chungnam	U302	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	13	33	4	10	5	28	KVCC-BA1300263	11.07.04.	Jeju	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	13	33	4	10	5	28	KVCC-BA1300264	11.07.06.	Chungnam	RDNC	AMP/GEN/NAL/STR	
22	15	33	4	9	6	28	KVCC-BA1300266	11.07.15.	Gyeongbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	15	33	4	11	5	28	KVCC-BA1300269	11.09.08.	Chungnam	RDNC	CHL/GEN/NAL/STR/TET	
22	14	33	2	11	5	28	KVCC-BA1300274	11.12.20.	Gyeongnam	U288	NAL/STR/TET	
22	14	33	2	11	5	28	KVCC-BA1300275	11.12.30.	-	U288	NAL/STR/TET	
22	14	33	4	11	5	28	KVCC-BA1400066	12.07.24.	Gyeonggi	U302	AMP/CHL/FOX/NAL/STR/TET	
22	14	33	4	11	6	28	KVCC-BA1400067	12.07.30	Gyeongnam	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	A (n=43)
22	15	33	4	11	5	28	KVCC-BA1400069	12.08.28.	Chungnam	RDNC	CHL/NAL/STR/SXT/TET	
22	13	33	4	10	5	28	KVCC-BA1400070	12.09.06.	Chungnam	RDNC	AMP/GEN/TET	
22	14	33	4	11	6	28	KVCC-BA1400071	12.09.19.	-	RDNC	AMP/CHL/GEN/NAL/STR/SXT	
22	14	33	4	9	5	28	KVCC-BA1400073	12.10.29.	Chungbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT	
22	14	33	4	9	5	28	KVCC-BA1400074	12.10.29.	Chungbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT	
22	15	33	4	11	5	28	KVCC-BA1400076	12.11.21.	Chungnam	U302	CHL/GEN/NAL/STR/SXT	
22	14	33	4	11	5	28	KVCC-BA1400077	12.12.04.	Gyeonggi	RDNC	AMP/CHL/NAL/STR/SXT	
22	14	33	4	10	5	28	KVCC-BA1400079	12.12.18.	Jeonbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT/TET	
22	16	33	4	7	5	28	KVCC-BA1300172	12.06.26.	Gyeongbuk	U288	CHL/NAL/STR/TET	
22	16	33	4	9	5	28	KVCC-BA1300270	11.10.17.	Chungbuk	RDNC	AMP/CHL/GEN/NAL/STR/TET	
22	13	33	4	7	5	28	KVCC-BA1300154	12.02.10.	Gyoengbuk	RDNC	AMP/GEN/TET	
22	10	33	4	10	3	28	KVCC-BA1400080*	12.12.27.	Gyeongnam	DT193	AMP/STR/TET	
22	10	33	4	12	4	28	KVCC-BA1300259	11.06.22.	Chungnam	U310	AMP/AUG/CEP/CHL/GEN/NAL/STR/SXT/TET	
22	12	33	4	12	5	28	KVCC-BA1300273	11.12.20.	Jeonbuk	RDNC	AMP/CHL/GEN/NAL/STR/TET	
22	9	33	4	12	5	28	KVCC-BA1300272	11.12.19.	Chungnam	RDNC	AMP/GEN/TET	
22	14	33	4	16	6	28	KVCC-BA1300155	12.02.16.	Gyeonggi	RDNC	AMP/CEP/CHL/NAL/STR/TET	
22	14	33	4	16	6	28	KVCC-BA1300164	12.05.10.	Jeonbuk	RDNC	AMP/CHL/STR/TET	
22	11	36	4	14	3	28	KVCC-BA1300254*	11.06.08.	Gyeonggi	RDNC	STR	
22	13	33	4	16	3	28	KVCC-BA1400072	12.10.19.	Chungbuk	RDNC	AMP/CHL/GEN/NAL/STR/SXT	
22	14	33	4	18	6	28	KVCC-BA1400075	12.11.09.	Chungnam	RDNC	AMP/CHL/STR/TET	
22	15	33	4	11	5	6	KVCC-BA1300258	11.06.21.	Gyeongnam	RDNC	NAL/STR/SXT/TET	B (n=1)
22	17	19	4	13	5	28	KVCC-BA1400068	12.08.24.	Gyeongnam	RDNC	AMP/NAL	C (n=1)
22	14	33	4	14	5	10	KVCC-BA1300170	12.06.01.	Gyoengbuk	RDNC	AMP/CHL/GEN/STR/TET	D (n=2)
22	14	33	4	15	5	24	KVCC-BA1300271	11.11.01.	Jeju	RDNC	AMP/AUG/CEP/CHL/STR/SXT/TET	
22	10	27	4	9	3	28	KVCC-BA1400078*	12.12.04.	Jeonbuk	RDNC	AMP/STR/TET	E (n=1)
25	8	36	4	8	2	8	KVCC-BA1300255*	11.06.10.	-	U291	-	F (n=2)
22	8	33	4	10	5	10	KVCC-BA1300265	11.07.15.	Chungnam	RDNC	AMP/CEP/CHL/GEN/NAL/STR/TET	

Fig. 1. MLVA profiles of *S.* Typhimurium that were analyzed using the BioNumerics program (Applied Maths) to generate a dendrogram based on the Dice coefficient. Fifty strains were divided into six clusters at similarity rates of 85%. **Salmenella* 4,5,12:i:- **RDNC: reacted, but did not conform. ***AMP, ampicillin; CEP, cephalothin; CHL, chloramphenicol; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline

BA1400080) were identified as *Salmonella* 4,[5],12:i:-, and had the O4 antigen, B serogroup and i antigen, but lacked the 1,2 antigen. The phage types of the four *Salmonella* 4,[5],12:i:- strains were U291, DT193 and RDNC (Fig. 1).

The antimicrobial resistance patterns of the 50 *S.* Typhimurium isolates are presented in Fig. 1. Of the 50 isolates, 47 were resistant to three antimicrobial agents in different classes, and 15 of these 47 isolates were pentaresistant to ampicillin, chloramphenicol, streptomycin, tetracycline and nalidixic acid. The higher level of antimicrobial resistance was observed among *S.* Typhimurium isolates from diarrheic pigs. Among the *Salmonella* 4,[5],12:i:- isolates, both KVCC-BA1400078 and KVCC-BA1400080 were resistant to ampicillin, streptomycin and tetracycline.

The flagellar gene expression data are shown in Table 2. All flagellar genes were present in 46 of the *S.* Typhimurium isolates (data not shown). However, the four *Salmonella* 4,[5],12:i:- isolates had different flagellar genes. One strain (KVCC-BA1400078) lacked all three flagellar genes (*fljA*, *fljB* and *hinA*) and three strains (KVCC-BA1300254, KVCC-BA1300255 and KVCC-BA1400080) had *hin,* but not *fljA* and *fljB*.

Figure 1 shows the MLVA profiles of the 50 *S.* Typhimurium isolates. The isolates were classified into 1 major cluster (A) and 5 minor clusters (B, C, D, E and F) based on 85% similarity in the MLVA, as follows: cluster A, 43 isolates (Chungnam: 14; Gyeongbuk: 8; Chungbuk: 6; Gyeonggi: 4; Gyeongnam: 3; Jeonbuk: 3; Jeonnam: 1; Jeju: 1; and unknown: 3); cluster B, 1 isolate (Gyeongnam); cluster C, 1 isolate (Gyeongnam); cluster D, 2 isolates (Gyeongbuk: 1;

Table 2. Distribution of flagella gene in *Salmonella* I 4,[5],12:i:-

KVCC No.	Date	Province	Gene of Salmonella						
			STM 2740	STM 2757	STM 1053-1997	STM 2773	*fljA*	*fljB*	*hin*
KVCC-BA1300254	2011.06.	Gyeonggi	+	+	+	–	–	–	+
KVCC-BA1300255	2011.06.	–	–	–	+	–	–	–	+
KVCC-BA1400078	2012.12.	Jeonbuk	+	+	–	+	–	–	–
KVCC-BA1400080	2012.12.	Gyeongnam	+	+	–	+	–	–	+

and Jeju: 1); cluster E, 1 isolate (Jeonbuk); and cluster F, 2 isolates (Chungnam: 1; unknown: 1). In this study, most of the *S.* Typhimurium isolates from Korean pigs were classified into cluster A, even though they were isolated from various locations nationwide. Among the *Salmonella* 4,[5],12:i:- isolates, KVCC-BA1300254 and KVCC-BA1400080 were classified into cluster A, while the remaining two strains, KVCC-BA1400078 and KVCC-BA1300255, were classified into clusters E and F, respectively.

In Korea, *S.* Typhimurium is well known as a causative agent of diarrhea in pigs [26]. *S.* Typhimurium was isolated from 13.9% (42/302) of the pigs examined during 2000–2006 and 22.3% (96/431) of the pigs examined during 2006–2007 [14, 25]. In this study, the prevalence of *S.* Typhimurium (7.19% in 2011 and 4.80% in 2012) was slightly lower than that of *S.* Typhimurium reported previously [14, 25]. However, recently, there have been many reports of *S.* Typhimurium variants isolated from humans and warm-blooded animals worldwide. *Salmonella* 4,[5],12:i:- is a variant of *S.* Typhimurium and an important pathogenic agent of food poisoning. According to the results of a surveillance project for *Salmonella* isolated from a pig slaughterhouse in Europe, *Salmonella* 4,[5],12:i:- has been increasing exponentially in Germany [10]. A recent report from the United States indicated that *Salmonella* 4,[5],12:i:- occurrences have increased exponentially in humans [7]. As a result of tracing *Salmonella* 4,[5],12:i:- in Korea, the serovar was isolated from humans in 2001 and from poultry in 2009–2011 [21, 22]. However, there were no reported isolates from pigs, despite much investigation. In this study, four strains of *Salmonella* 4,[5],12:i:- were isolated from 896 pigs between 2011 and 2012. Expression of the *fljAB* operon (*fljA*, *fljB* and *hin*) in *Salmonella* 4,[5],12:i:- varies from country to country [27]. The four *Salmonella* 4,[5],12:i:- strains isolated in this study exhibited flagellar gene profiles that have not yet been reported in Korea. One strain in particular, KVCC-BA1400078, had high identity to a *Salmonella* 4,[5],12:i:- clone isolated from a human in the United States in the PCR screens to contain flagella gene [27]. It is difficult to determine whether *Salmonella* 4,[5],12:i:- existed previously in swine in Korea or was brought in from outside the country; however, the emergence of *Salmonella* 4,[5],12:i:- isolates in Korean pigs suggests an urgent need for the establishment of a strict Hazard Analysis and Critical Control Point program in the process of importing animals and farming animals.

More than 90% of the isolates obtained in 2006–2007 exhibited antimicrobial resistance to streptomycin, tetracycline and sulfamethoxazole [25]. To avoid the misuse of antimicrobial agents in livestock and protect public health through safe management, the use of antimicrobial agents in livestock decreased steadily, beginning in July 2011, until it was finally banned in March 2013 in accordance with Korean policy. Compared to previous studies, the prevalence of antimicrobial resistance decreased slightly in this study [24, 25, 27]. In terms of the distribution of phage types among *Salmonella* 4,[5],12:i:- isolates from several European countries, most strains are of phage type DT193 or DT120, and these strains are multidrug-resistant [13]. In Korea, almost all *S.* Typhimurium and *Salmonella* 4,[5],12:i:- isolates exhibited ampicillin, streptomycin and tetracycline resistance. In particular, *Salmonella* 4,[5],12:i:- strain KVCC-BA1400080 (phage type DT193) was resistant to ampicillin, streptomycin and tetracycline, similar to phage type DT193 strains isolated in Europe [13]. Accordingly, antimicrobial susceptibility monitoring of *Salmonella* isolates from pigs is needed for appropriate prevention and treatment.

MLVA exhibited sufficient discriminatory power in both *Brucella* isolates from cattle and *S.* Gallinarum isolates from chickens in Korea [15, 17]. However, by MLVA alone, *Salmonella* 4,[5],12:i:- cannot be distinguished from the *S.* Typhimurium cluster, because of the close genetic relationship between the two serovars. However, profiles created by MLVA further classified the causative agents from each outbreak.

In conclusion, *S.* Typhimurium is commonly isolated from pigs presenting with diarrhea, and this is the first reported detection of *Salmonella* 4,[5],12:i:- from pigs in Korea. The isolation of *Salmonella* 4,[5],12:i:- is increasing worldwide, and the number of antimicrobial-resistant strains is increasing steadily. Therefore, continuous monitoring at the national level is necessary for food poisoning tracking and pig disease analysis.

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