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Molecular subtyping and antimicrobial susceptibility of *Streptococcus dysgalactiae* subspecies *equisimilis* isolates from clinically diseased pigs

Sang-Ik Oh [1,2,†], **Jong Wan Kim** [1], **Jongho Kim** [1], **Byungjae So** [1], **Bumseok Kim** [2], **Ha-Young Kim** [1,*]

[1]Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon 39660, Korea
[2]College of Veterinary Medicine, Jeonbuk National University, Iksan 54596, Korea

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***Corresponding author:**
Ha-Young Kim
Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon 39660, Korea.
E-mail: kimhy@korea.kr

[†]Present address: Division of Animal Diseases & Health, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

ORCID iDs
Sang-Ik Oh
https://orcid.org/0000-0003-0877-9170
Jong Wan Kim
https://orcid.org/0000-0002-8758-2300
Jongho Kim
https://orcid.org/0000-0002-3719-860X
Byungjae So
https://orcid.org/0000-0002-6125-6873
Bumseok Kim
https://orcid.org/0000-0003-0392-2513
Ha-Young Kim
https://orcid.org/0000-0002-1332-3832

ABSTRACT

Background: *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) acts as an etiological agent for lameness, neurological signs, and high mortality in pigs. Despite its importance in pig industries and zoonotic potential, little is known about the effects of this pathogen.
Objectives: This study aimed to determine the molecular characteristics and antimicrobial resistance of SDSE strains isolated from diseased pigs.
Methods: A total 11 SDSE isolates were obtained from diseased pigs. Bacterial identification, PCR for virulence genes, *emm* typing, and antimicrobial resistance genes, multilocus sequence typing, and antimicrobial susceptibility test were performed.
Results: Nine isolates were from piglets, and 8 showed lameness, sudden death, or neurological signs. The isolates were PCR-positive for *sla* (100%), *sagA* (100%), and *scpA* (45.5%), and only 1 isolate amplified the *emm* gene (*stL2764*). Eight different sequence types were detected, categorized into 2 clonal complexes and 4 singletons. All the isolates in this study were included in a small cluster, which also contained other strains derived from humans and horses. The minimum inhibitory concentrations for the tested beta-lactams were low, while those for macrolides, tetracyclines, and fluoroquinolones were relatively high. PCR analysis of the macrolide and tetracycline resistance genes demonstrated that the isolates carried *erm*(B) (18.2%, n = 2), *mef*(A/E) (9.1%, n = 1), *tet*(M) (18.2%, n = 2), and *tet*(O) (90.2%, n = 10). Two isolates presented a mutation in *parC*, which is associated with fluoroquinolone resistance.
Conclusion: This study provided insight into swine-derived SDSE, as it is related to veterinary medicine, and elucidated its zoonotic potential, in the context of molecular epidemiology and antimicrobial resistance in public health.

Keywords: Drug resistance; multilocus sequence typing; *Streptococcus dysgalactiae* subspecies *equisimilis*; swine; virulence

INTRODUCTION

Streptococcus dysgalactiae subspecies *equisimilis* (SDSE), which is a pyogenic pathogen belonging to Lancefield groups C, G, A, or L, has been recovered increasingly from severe invasive human infections worldwide [1,2]. SDSE infection in humans can cause a broad spectrum of diseases,

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Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contribution

Conceptualization: Oh SI, Kim HY; Data Curation: Oh SI, Kim J, Kim HY; Formal analysis: Oh SI; Funding acquisition: Kim JW, So B; Investigation: Oh SI, Kim HY; Methodology: Oh SI, Kim J, Kim HY; Project administration: Kim JW, So B; Resources: Kim HY; Software: Oh SI; Supervision: Kim HY, Kim B; Validation: Kim HY; Visualization: Oh SI; Writing - original draft: Oh SI; Writing - review & editing: Kim HY, Kim B.

including skin abscess, cellulitis, pharyngitis, arthritis, bacteremia, endocarditis, and toxic shock syndrome [1]. Due to the severity of SDSE infection in humans, studies for the molecular characterization and zoonotic potential of this bacterium derived from animal hosts are of great interest [2-6]. However, most studies on SDSE isolated from animals have been limited to horses, and only few studies have addressed swine-derived SDSE isolates [7-10].

Although SDSE is a member of the normal flora in pigs, it is considered an important beta-hemolytic *Streptococcus*, especially in piglets [11]. Vaginal secretions and milk from postparturient sows and contaminated floor surfaces, which are abrasive and harmful to the feet, are the most likely sources of infection in pigs [11,12]. Our recent findings highlighted the importance of management of floor surfaces in farrowing pens, especially for neonatal piglets [10]. SDSE infection in pigs could cause septicemia, arthritis, endocarditis, or meningitis, which extensively overlap with the symptoms of SDSE infection in humans [11,13]. Previous studies have suggested that the incidence and severity of invasive SDSE infections in humans have some relationship with several bacterial virulence factors, whereas the relationship in pigs is yet to be investigated [13]. As recent studies warn that SDSE may have the potential of zoonotic infections, the possibility of pig-to-human infection could increase in individuals who work in direct contact with pig or raw meat [5,6]. Therefore, it is vital to elucidate the possibility of SDSE crossing the pig-to-human interspecies barrier using epidemiological approaches. In addition, pigs with a streptococcal infection, including SDSE infections, are widely treated by antimicrobial therapy [11]. Given that SDSE isolates from pigs may be zoonotic pathogens, it is important to survey their antimicrobial resistance genotypes and phenotypes to prepare for a potential public health hazard.

Here, we present the analysis of clinical features, molecular characteristics, and antimicrobial resistance of SDSE isolates from clinically diseased pigs. Through this study, we provide valuable information to improve awareness and understanding of SDSE infection in pigs and its zoonotic potential.

MATERIALS AND METHODS

Samples and bacterial isolation

In total, 11 SDSE isolates were obtained from clinically diseased pigs, which were submitted to the Animal and Plant Quarantine Agency of Korea for disease diagnosis from 2008 to 2017. The clinical features of these cases are summarized in **Table 1**. Briefly, the pigs were aged 3–70 days, with an average age of 32.5 days. Most cases (8 cases, 72.7%) showed at least one of the following clinical signs: lameness, neurological signs, or sudden death. Bacterial culture and virus detection were performed on the selected samples corresponding to the clinical symptoms of each case. Suspected beta-hemolytic *Streptococcus* grown on blood agar plate from each case were recovered from lungs (n = 5), joints (n = 4), pericardium (n = 1), and peritoneal cavity (n = 1).

Bacterial identification

Candidate beta-hemolytic isolates were identified as SDSE using the VITEK 2 system (GP ID card; bioMérieux, France) and confirmed by 16S ribosomal RNA (rRNA) gene sequencing according to a previous study [14]. Lancefield serological group was determined for groups A, B, C, and G by a Strep Grouping Kit (Denka Seiken, Japan).

Table 1. Clinical features of the swine cases in this study

Case No.	Age (day)	Year of case submission	Source of SDSE isolation	Other pathogens detected (sample)	Clinical signs of cases				
					LM	NS	SD	MM	RS
1	3	2016	Peritoneal cavity	ND	+			+	
2	5	2015	Joint	ND	+			+	
3	10	2013	Joint	ND	+	+			
4	10	2015	Lung	ND			+		
5	20	2010	Lung	PM, SSUIS, PCV2 (lung)		+	+		
6	40	2017	Joint	PM, HPS, MHR (lung)					+
7	45	2008	Lung	PM, PRRSV, PCV2 (lung)					+
8	45	2014	Joint	ND	+	+	+		
9	50	2008	Lung	PM (lung)		+	+	+	
10	60	2010	Lung	HPS, MHR (lung)		+	+		+
11	70	2013	Pericardium	PM (nasal concha)					+

SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*; ND, not detected; HPS, *Haemophilus parasuis*; MHR, *Mycoplasma hyorhinis*; PCV2, porcine circovirus type 2; PM, *Pasteurella multocida*; PRRSV, porcine reproductive and respiratory syndrome virus; SSUIS, *Streptococcus suis*; LM, lameness; NS, neurological sign; SD, sudden death; MM, mass mortality; RS, respiratory symptom.

Polymerase chain reaction (PCR) assay for virulence genes and *emm* typing

To increase the sensitivity of PCR detection, pure SDSE isolates were inoculated in Todd-Hewitt broth (THB; Difco, USA) at 5% CO_2, 37°C for 24 h. Briefly, 300 μL of incubated THB was pelleted by centrifugation at 10,600 g for 2 min, and the supernatant was discarded. Further, the pellet was incubated at 37°C for 30 min in 50 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 10 μL of mutanolysin (3,000 U/μL, Sigma, USA), and 8 μL of hyaluronidase (30 mg/mL, Sigma). Subsequently, DNA extraction and purification were conducted using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instruction.

Presence of superantigenic (SAg) genes (*speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa*, *smez*, and $speG^{dys}$) and other specific virulence genes (*sla*, *sagA*, *scpA*, *slo*, and *ska*) in the genome of the isolates were identified using previously published primers and protocols [15].

The *emm* typing was carried out using primers and conditions set by the Centers for Disease Control and Prevention (CDC) guidelines (https://www.cdc.gov/streplab/protocol-emm-type.html). The obtained sequences were compared to the sequences in the *emm* type database available on the CDC website (http://www2a.cdc.gov/ncidod/biotech/strepblast.asp).

Multilocus sequence typing (MLST)

MLST was performed by sequencing for 7 housekeeping genes (*gki*, *gtr*, *murI*, *mutS*, *recP*, *xpt*, and *atoB*). Unique sequences at each locus were assigned allele numbers, and the corresponding combination of the 7 allele numbers for each isolate was used to define the sequence type (ST) using the PubMLST database (http://pubmlst.org/sdysgalactiae/). Clonal complexes (CCs) were defined at the single-locus variant (SLV) level. Comparison among pigs and other hosts' isolates was achieved through analysis of previously published MLST data [2,5,6,16-18]. An expansion of the goeBURST algorithm in Phyloviz software was used to generate the compilation of the minimum spanning tree (MST), reflecting possible relationships among pig-, dog-, horse-, and human-derived STs.

Antimicrobial susceptibility test

The minimum inhibitory concentration (MIC) was determined by the standard microbroth dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI VET01-A4, 2013) using Sensititre Standard Susceptibility MIC Plates BOPO6F (TREK

Diagnostic Systems, USA). *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 were used as the quality control strains. Overall, 50% MIC (MIC_{50}) and 90% MIC (MIC_{90}) were determined for each antimicrobial agent.

Identification of antimicrobial resistance genes

Presence of genes commonly associated with erythromycin resistance (*erm*(A), *erm*(B), and *mef*(A/E)) and tetracycline resistance (*tet*(K), *tet*(L), *tet*(M), and *tet*(O)) was assessed by PCR using previously published primers and protocols [19]. Segments of *gyrA* and *parC* containing the quinolone resistance determining regions (QRDR) were amplified and sequenced using primers, as previously described [20]. A comparative analysis of sequences was conducted using MEGA software, version 5.0 (http://www.megasoftware.net). ClustalW was used to perform multiple alignments of the nucleotide or predicted amino acid sequences. The reference sequences of *gyrA* and *parC* (SDSE strain AC-2713, GenBank accession number: HE858529) were obtained from GenBank.

RESULTS

SDSE isolate identification

The characteristics of the 11 swine-derived SDSE isolates are shown in **Table 2**. Sequences obtained from the isolates using 16S rRNA sequencing presented 93.5%–100% similarity to the SDSE reference strain (GenBank accession number: CP002215). All 16S rRNA gene sequences (n = 11) were deposited in the GenBank database (accession numbers: KY986687–KY986697). All the isolates presented as Lancefield group C antigens.

Distribution of virulence genes and *emm* typing

All the isolates showed an identical virulence gene profile, except for *scpA* gene; PCR-negative for *slo*, *ska*, and all the tested SAg genes (*speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa*, *smez*, and $speG^{dys}$), while PCR-positive for *sla* and *sagA*. However, only 5 isolates (45.5%) carried the *scpA* (**Table 2**). Among the 11 isolates, *emm* gene was successfully amplified in only 1 isolate from case number 1 (KY986697), which typed as *stL2764* (**Table 3**).

MLST analysis

MLST performed for all the 11 SDSE isolates yielded 8 STs. Among them, 6 isolates included previously published STs, specifically ST315 (n = 3, 27.3%), ST252 (n = 2, 18.2%), and ST234

Table 2. Phenotypic and genotypic characteristics of SDSE isolates from clinically diseased pigs

Case No.	Lancefield group	Similarity to 16S rRNA sequence of SDSE type strain (%)	GenBank accession No.	Virulence gene profile					
				sla	*sagA*	*scpA*	*slo*	*ska*	SAg genes*
1	C	97.5	KY986697	+	+	+	-	-	-
2	C	99.9	KY986688	+	+	+	-	-	-
3	C	99.9	KY986687	+	+	-	-	-	-
4	C	99.3	KY986693	+	+	+	-	-	-
5	C	99.9	KY986694	+	+	-	-	-	-
6	C	98.5	KY986696	+	+	-	-	-	-
7	C	95.0	KY986689	+	+	-	-	-	-
8	C	93.5	KY986695	+	+	-	-	-	-
9	C	97.0	KY986690	+	+	+	-	-	-
10	C	98.6	KY986691	+	+	+	-	-	-
11	C	100	KY986692	+	+	-	-	-	-

SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*; rRNA, ribosomal RNA; SAg, superantigenic.
*SAg genes: *speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa*, *smez*, and $speG^{dys}$.

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Table 3. Molecular epidemiological characteristics of SDSE isolates from clinically diseased pigs

Case No.	*emm* type	MLST scheme		
		Allelic profile*	ST	CC
1	*stL2764*	30-13-6-30-12-13-33	**338**	Singleton
2	NT	30-13-6-6-12-56-33	252	252/339
3	NT	32-13-28-30-39-48-29	315	315/336
4	NT	30-13-6-**41**-12-56-33	**339**	252/339
5	NT	32-13-28-28-39-48-29	**336**	315/336
6	NT	30-13-6-27-39-**71**-33	**340**	Singleton
7	NT	32-13-28-30-39-48-29	315	315/336
8	NT	32-13-6-27-39-48-33	**337**	Singleton
9	NT	32-33-27-27-39-48-29	234	Singleton
10	NT	30-13-6-6-12-56-33	252	252/339
11	NT	32-13-28-30-39-48-29	315	315/336

SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*; MLST, multilocus sequence typing; ST, sequence type; CC, clonal complex; NT, non-typable.
*Allelic profile, *gki-gtr-murI-mutS-recP-xpt-atoB*.
Novel alleles and STs are marked in bold.

(n = 1, 9.1%) (**Table 3**). Five other isolates were identified as novel STs, which were assigned as ST336, ST337, ST338, ST339, and ST340. MLST sequences alignment revealed 2 novel sequences in *mutS* and *xpt*, which were designated as *mutS*41 and *xpt*71, respectively (**Table 3**). The STs were categorized into 2 CCs and 4 singletons at the SLV level. Among them, CC315/336 was the most prevalent (n = 4, 36.4%), followed by CC252/339 (n = 3, 27.3%). The MST, showing the relationships between STs, indicated that most STs were categorized according to their host of origin (**Fig. 1**). Three clusters (cluster A, B, and C) could be defined corresponding to a difference of at least 6 different loci. Cluster A and C consisted almost exclusively of human and horse isolates, respectively, from previous studies [2,5,6,16-18]. Cluster B was composed of the swine-derived isolates from this study (n = 11) and human- (n = 4) and horse-derived (n = 5) isolates from previous studies [2,16].

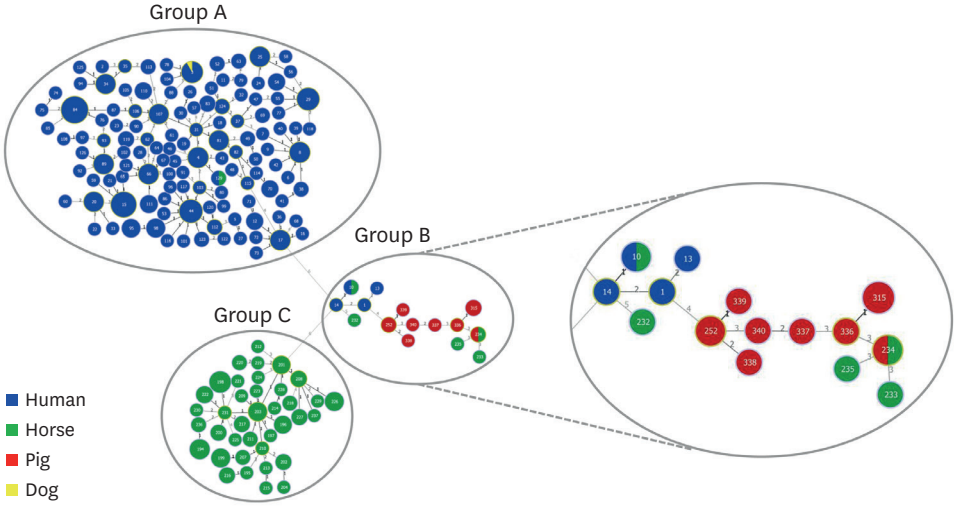

Fig. 1. Minimum spanning tree based on sequence types of 494 *Streptococcus dysgalactiae* subspecies *equisimilis* strains isolated from humans, horses, pigs, and dogs. The size of each circle is proportional to the number of isolates with that particular sequence type on a logarithmic scale. Numbers on branches represent the number of loci that differ from the founder sequence type.

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Table 4. MIC value distribution, MIC_{50}, and MIC_{90} of SDSE strains from clinical diseased pigs against the tested antimicrobials

Case No.	MIC (μg/mL)									
	Beta-lactams			Macrolides			Tetracyclines		Fluoroquinolones	
	TIO	PEN	AMP	TYL	TIL	TUL	CTE	OTE	DAN	ENR
1	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	2
2	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	1
3	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	1
4	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	2
5	≤ 0.25	≤ 0.12	≤ 0.25	≤ 0.5	≤ 4	16	> 8	> 8	> 1	2
6	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	> 2
7	≤ 0.25	≤ 0.12	≤ 0.25	≤ 0.5	≤ 4	32	> 8	> 8	> 1	2
8	≤ 0.25	≤ 0.12	≤ 0.25	≤ 0.5	≤ 4	16	> 8	> 8	> 1	2
9	≤ 0.25	≤ 0.12	≤ 0.25	> 32	32	64	> 8	> 8	> 1	2
10	0.5	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	1
11	≤ 0.25	≤ 0.12	≤ 0.25	≤ 0.5	≤ 4	16	> 8	> 8	> 1	2
MIC_{50}	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	2
MIC_{90}	≤ 0.25	≤ 0.12	≤ 0.25	> 32	> 64	> 64	> 8	> 8	> 1	2

SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*; MIC, minimum inhibitory concentration; TIO, ceftiofur; PEN, penicillin; AMP, ampicillin; TYL, tylosin; TIL, tilmicosin; TUL, tulathromycin; CTE, chlortetracycline; OTE, oxytetracycline; DAN, danofloxacin; ENR, enrofloxacin.

Antimicrobial susceptibility of SDSE isolates

The MIC value distribution, MIC_{50}, and MIC_{90} of the SDSE isolates against 10 tested antimicrobials are shown in **Table 4**. Beta-lactams (ampicillin, ceftiofur, and penicillin) showed the lowest MIC values for all the isolates, while tetracyclines (chlortetracycline and oxytetracycline) and danofloxacin presented the highest MIC values in the tested range of concentrations, for all the isolates. The MIC_{50} and MIC_{90} values of the isolates were identical for each tested antimicrobials in this study.

Identification of antimicrobial resistance genes

The distribution of macrolide and tetracycline resistance genes, as well as mutation in QRDR of GyrA and ParC, encoded by *gyrA* and *parC*, respectively, is shown in **Table 5**. Only 1 (9.1%) and 2 (18.2%) macrolide-resistant isolates contained *mef*(A/E) and *erm*(B), respectively. No isolate contained *erm*(A). Among the tested tetracycline resistance genes, *tet*(M) and *tet*(O) were found in 2 (18.2%) and 10 isolates (90.9%), respectively. No isolates had substitutions in GyrA, and only 2 isolates showed substitution in ParC: Glu96 to Asp and Ser79 to Phe.

Table 5. Distribution of antimicrobial resistance genes and *gyrA*, *gyrB* mutation of SDSE isolates from clinically diseased pigs

Case No.	Antimicrobial resistance gene							Amino acid substitution in	
	Macrolides			Tetracyclines				Fluoroquinolones*	
	erm(A)	*erm*(B)	*mef*(A/E)	*tet*(K)	*tet*(L)	*tet*(M)	*tet*(O)	*gyrA*	parC
1	-	+	-	-	-	-	+	-	-
2	-	-	-	-	-	+	+	-	Glu96 to Asp
3	-	-	-	-	-	-	+	-	-
4	-	-	-	-	-	-	+	-	-
5	-	-	-	-	-	-	+	-	-
6	-	+	-	-	-	-	-	-	Ser79 to Phe
7	-	-	-	-	-	-	+	-	-
8	-	-	-	-	-	-	+	-	-
9	-	-	+	-	-	-	+	-	-
10	-	-	-	-	-	+	+	-	-
11	-	-	-	-	-	-	+	-	-

SDSE, *Streptococcus dysgalactiae* subspecies *equisimilis*.
*Amino acid substitution in parC of SDSE isolates resistant to fluoroquinolone compared to the reference SDSE AC-2713 (GenBank accession number: HE85852).

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DISCUSSION

SDSE is known to cause sporadic septicemia and arthritis in suckling pigs, endocarditis in growing pigs, and ascending uterine infection in sows [10,21]. As for age distribution, most SDSE-infected pigs in this study were suckling (≤ 21-day-old, n = 5) or weaning (≤ 60-day-old, n = 5) piglets. The result is consistent with a previous study suggesting that SDSE infection is usually seen in piglets [11]. Interestingly, no other systemic infectious pathogen, except for SDSE, was found in piglets below 10 days of age, implying that even a single SDSE infection can lead to sudden death or lameness in neonatal piglets.

All the isolates in this study belonged to the Lancefield group C. Although Katsumi et al. [22] reported that only 59.0% of SDSE strains isolated from slaughtered pigs belonged to Lancefield group C, our finding showed a trend similar to that of other previous studies, wherein group C was the most predominant strain in pigs [22,23]. Conversely, the majority of human-recovered SDSE isolates from previous studies have been categorized as Lancefield group G [15,24]. This discrepancy could be due to the heterogeneity of streptococci possessing Lancefield group C and G cell wall carbohydrates, in regards to the predilection for host species and clinical diseases produced in animals and humans [25].

In this study, the distribution of virulence genotype obtained from swine SDSE isolates was inconsistent in comparison with that from previous studies on human SDSE strains, which harbored some of the SAg genes [15,26-28]. Further, most human SDSE strains in previous studies contained *slo* and *ska*, which were not found in this study [15,26-29]. Notably, all the swine isolates in this study harbored the *sla* gene, which is associated with epithelial cells, while this gene was not found in human-derived SDSE isolates from previous studies [15,25,28]. This suggests that SDSE could transfer between humans and animals to acquire specific virulence genes for binding to epithelial cells in each host [3]. Considering the zoonotic potential of SDSE, virulence genes in animal-derived strains should be closely monitored because the pathogen, which showed similarity with *Streptococcus pyogenes*, might cause serious human infections in the near future. Furthermore, the challenge trials of SDSE strains in pigs are needed to improve our understanding of this bacterium and to determine which virulence genes play a role in pathogenesis.

The M protein, encoded by *emm* gene, is also an important virulence factor, which prevents phagocytosis in the host [1]. Many studies have used *emm* typing for epidemiological investigation of SDSE isolates from humans [17,18,24,30]. Some reports suggested that certain *emm* types from human SDSE strains are associated with antimicrobial resistance, while others are associated with severe infection [14,24,31]. However, this hypothesis may be applicable only for SDSE isolates from human sources, given that the isolates from animal sources are often *emm* non-typable [2]. Although our study could not find any associations due to typing of only 1 isolate, the amplified type (*stL2764*) in this study has already been documented in several surveys of the pig, human, and horse SDSE [2,24,32,33]. Among them, Pinho et al. [2] strongly doubted that the horse isolate identified as *stL2764* had zoonotic potential.

To elucidate swine potential for zoonotic transmission, the isolates were characterized using the MLST scheme. Our data showed that SDSE isolates from pigs in Korea had low genetic diversity, since most STs were linked at the SLV level and all were related at the triple-locus-variant level. MST analysis showed that all STs from pigs in this study were included in cluster

B, which was separated from cluster A and C by a difference of at least 6 different loci. These findings indicated the existence of genetically related strains (included in cluster B), which could be isolated from humans, horses, and pigs. Taken together, these results suggest that swine-derived SDSE strains, all belonging to MLST-defined cluster B, have the potential to be zoonotic pathogens. However, the sample size of this study was too small to draw a general conclusion. Therefore, further studies with large numbers of SDSE isolates from both pigs and other diverse animal hosts are required to verify the infectious capacity of swine-derived SDSE strains in a wide range of hosts, including humans or a host-specific genomovar among SDSE from animals.

In the absence of effective vaccination against groups C or L streptococci, appropriate and accurate usage of antimicrobials has enabled veterinarians to combat SDSE infection in pigs [11]. Despite the limited information about antimicrobial susceptibility of swine-derived isolates, a recent study presented alarming MIC_{50} and MIC_{90} for most tested antimicrobials [9]. The results of this study confirmed beta-lactams as the best choice for treatment, which is consistent with previous studies in pigs and humans [9,11,15]. In this study, macrolides, tetracyclines, and danofloxacin showed the highest MIC_{50} and MIC_{90} values among the tested dilution ranges, suggesting these antimicrobials were not suitable for treating SDSE-infected pigs. These results were also consistent with a previous study on swine-derived SDSE from Brazil, yet inconsistent with human-derived SDSE from Korea, which showed the lowest MIC_{50} value against erythromycin (≤ 0.12 μg/mL) [9,31]. Therefore, our findings serve as a crucial warning for public health because humans showing tonsillopharyngitis as a result of SDSE infection, who are allergic to beta-lactams, are usually treated with macrolides as an alternative antimicrobial therapy; if this treatment does not have positive response for the patients, tetracyclines and fluoroquinolones should be considered as a second choice [34].

The resistance genes of macrolides, tetracyclines, and fluoroquinolones were examined to investigate the genetic variation. To the best of our knowledge, there have been no previous attempts to survey antimicrobial resistance genes of SDSE from animal-species, despite the risk of possible transfer of resistant strains from animals to humans. We found that the results of this study (*erm*(A): 0%, *erm*(B): 18.2%, and *mef*(A/E): 9.1%) were similar to that of a previous study on human-recovered SDSE in Korea (*erm*(A): 4.3%, *erm*(B): 20.3%, and *mef*(A): 8.7%), which differed from previous studies from China (*erm*(A): 0%, *erm*(B): 78.6%, and *mef*(A/E): 5.4%) and Japan (*erm*(A): 15.5%, *erm*(B): 11.3%, and *mef*(A): 2.8%) [15,30]. Of tetracycline resistance genes, the results (*tet*(M): 18.2%, *tet*(O): 90.9%) differed from previous studies in China and Korea, wherein the *tet*(M) gene (China: 73.2% and Korea: 29.0%) was more predominant than the *tet*(O) gene (China: 5.4% and Korea: 1.4%) in human-derived SDSE isolates [15,30]. Mutations in the QRDR of *gyrA* or *parC* are known as a major mechanism of fluoroquinolone resistance. In this study, 2 isolates had a single amino acid substitution in ParC: Glu96 to Asp and Ser79 to Phe. Notably, a position mutation at Ser79 to Phe in *parC* has been found frequently in SDSE from humans [15,30]. Given that the mutations in both *gyrA* and *parC* are associated with high-level resistance against fluoroquinolones, continuous monitoring of the QRDR in swine-derived SDSE isolates is needed to detect emerging fluoroquinolone resistant strains [15]. The discrepancy between the observed phenotypic and genotypic antimicrobial resistance in this study could be explained by a previous study, which suggested that the possession of antimicrobial resistance genes does not always accurately reflect the bacterial resistance phenotype [35]. Taken together, SDSE isolates from clinically diseased pigs had only slightly different distribution of antimicrobial resistance genes of macrolides, tetracyclines, and

fluoroquinolones from that of humans. Thus, more prudent use of antimicrobials, especially macrolides, tetracyclines, and fluoroquinolones, is recommended in veterinary medicine.

In conclusion, our findings suggest that SDSE should be considered as an important pathogen in piglets, which could cause lameness, neurological signs, and sudden death. Analysis of virulence genes differed from previous studies on human isolates in distribution of *sla*, *slo*, and *ska* genes. Based on our epidemiological studies, we provide evidence supporting the previously proposed hypothesis that several specific SDSE may have zoonotic potential. Moreover, SDSE from pigs in this study presented alarming MIC values for macrolides, tetracyclines, and fluoroquinolones, which are often used as alternatives to beta-lactams in human-infected SDSE. Thus, further studies with a large number of strains are essential to clarify the zoonotic potential of SDSE from diverse animal populations.

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