



Prevalence of Complement-Mediated Cell Lysis-like Gene (*sicG*) in *Streptococcus dysgalactiae* subsp. *equisimilis* Isolates From Japan (2014–2016)

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Background: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE; a β -hemolytic streptococcus of human or animal origin) infections are emerging worldwide. We evaluated the clonal distribution of complement-mediated cell lysis-like gene (*sicG*) among SDSE isolates from three central prefectures of Japan.

Methods: Group G/C β -hemolytic streptococci were collected from three institutions from April 2014 to March 2016. Fifty-five strains (52 from humans and three from animals) were identified as SDSE on the basis of 16S rRNA sequencing data.; they were obtained from 25 sterile (blood, joint fluid, and cerebrospinal fluid) and 30 non-sterile (skin-, respiratory tract-, and genitourinary tract-origin) samples. *emm* genotyping, multilocus sequence typing, *sicG* amplification/sequencing, and random amplified polymorphic DNA (RAPD) analysis of *sicG*-positive strains were performed.

Results: *sicG* was detected in 30.9% of the isolates (16 human and one canine) and the genes from the 16 human samples (blood, 10; open pus, 3; sputum, 2; throat swab, 1) and one canine sample (open pus) showed the same sequence pattern. All *sicG*-harboring isolates belonged to clonal complex (CC) 17, and the most prevalent *emm* type was *stG6792* (82.4%). There was a significant association between *sicG* presence and the development of skin/soft tissue infections. CC17 isolates with *sicG* could be divided into three subtypes by RAPD analysis.

Conclusions: CC17 SDSE harboring *sicG* might have spread into three closely-related prefectures in central Japan during 2014–2016. Clonal analysis of isolates from other areas might be needed to monitor potentially virulent strains in humans and animals.

Key Words: *Streptococcus dysgalactiae* subsp. *equisimilis*, Complement-mediated cell lysis-like gene, Clonal complex, *emm* genotype, Skin and soft tissue infections, Random amplified polymorphic DNA analysis, Japan

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INTRODUCTION

In 1996, Vandamme *et al* [1] proposed that the new streptococcus subspecies, *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE), detected among human- and animal-origin streptococ-

cal isolates, is a clinically relevant pathogen. This microorganism possesses Lancefield group G/C antigens (and rarely group A antigen), exhibits strong β -hemolytic activity and large colony size, and exerts streptokinase activity on human plasminogen and proteolytic activity on human fibrin. The *S. anginosus* group

is a distinct category of group G/C streptococci, consisting of *S. anginosus*, *S. constellatus*, and *S. intermedius*. This *S. anginosus* group is easily distinguished from SDSE on the basis of biochemical characteristics and colony size on sheep blood agar. Since 2000, severe invasive infections (e.g., streptococcal toxic shock syndrome [STSS], necrotizing fasciitis, meningitis, infectious endocarditis, septic arthritis, and osteomyelitis) due to SDSE have been increasingly reported among elderly patients. The current worldwide medical conditions [2–6] are similar to those caused by group A streptococci (GAS, *S. pyogenes*) and group B streptococci (GBS, *S. agalactiae*). In addition, neonatal STSS and the disease burden caused by SDSE among schoolchildren have also been examined [7, 8]. Interestingly, sepsis with unknown foci is more frequent among GBS-infected patients than among SDSE- or GAS-infected patients, while cellulitis is less frequent among GBS-infected patients than among SDSE- or GAS-infected patients [6]. SDSE-infected patients present more often with septic arthritis than GBS-infected patients, whereas GAS-infected patients exhibit abscesses involving deeper than skin sites more often than SDSE-infected patients [6].

The *emm* gene encodes the filamentous surface M protein, which plays a role in attachment to host epithelial cells and exerts anti-phagocytosis activity against immune cells. The M protein is used for typing GAS and SDSE isolates in epidemiological investigations. Of the 229 isolates with *emm* genotypes investigated by Takahashi *et al* [9], 55 (24%) had the *stG6792* type, which was strongly associated with poor patient outcomes.

A number of GAS strains express related secretory proteins: streptococcal inhibitor of complement (SIC) and the distantly related SIC (DRS) variant. SIC is found in only two GAS *emm* types (*emm1* and *emm57*) and is thought to be a ligand for complement proteins C6 and C7, inhibiting the formation of the membrane attack complex, thereby blocking complement-mediated lysis [10, 11]. Similar to GAS, the streptococcal inhibitor of complement-mediated cell lysis-like gene (*sicG*), which encodes a newly discovered extracellular virulence factor, DrsG, is harbored by only a few *emm* type SDSE strains [12]. Interestingly, the protein encoded by *sicG* is reported to suppress the antimicrobial peptide LL-37, which is expressed in sweat as a part of the skin's innate defense system [13], suggesting the potential for cutaneous colonization by SDSE.

To date, only a few studies have examined the relationship between the presence of *sicG* and molecular epidemiological markers (e.g., *emm* genotypes, sequence types [STs], and antimicrobial resistance determinants) in SDSE isolates [14]. We aimed to determine the association between the presence of

sicG and the microbiological and clinical features of SDSE isolates collected from three central prefectures (Saitama, Tokyo, and Chiba) in Japan over two years (2014–2016). At present, many people in Japan (from children to the elderly) have companion animals (dogs and cats) in their household. In addition, a number of hospitals have introduced animal-assisted therapy as a part of their mental care of patients. Therefore, we evaluated the molecular characteristics of SDSE strains from these animals.

METHODS

1. Collection of streptococcal isolates and host information

A total of 64 group G/C β -hemolytic streptococci isolates were selected from the databases of three institutions (two hospitals and one laboratory center located in Saitama, Tokyo, and Chiba) from April 2014 to March 2016. Two veterinary clinicians kindly provided stored group G streptococcal isolates from companion animal infection sites. Each of the strains (one strain per host) was inoculated onto a sheep blood agar plate, which was incubated under 5% CO₂ at 35°C for 24 hr. Gray-white colonies with β -hemolytic activity were subjected to a latex agglutination test with specific antisera for the classification of Lancefield antigens (Seroiden Strepto kit, Eiken Chemical Co., Ltd., Tokyo, Japan). All isolates were stored at –70°C to –80°C until being processed for further evaluation. Group G strain D166B (ATCC 12394) was used as a quality control [15].

Patient information (including underlying medical conditions, clinical diagnoses, blood test data for bacterial cultures, therapeutic antimicrobial agents, and outcomes) concerning the presence of invasive or noninvasive diseases was retrieved from their medical charts. Invasive infections were defined as the isolation of SDSE from a sterile site (blood, joint fluid, or cerebrospinal fluid) [9], while noninvasive infections were defined as the isolation of SDSE from a nonsterile site (skin-, respiratory tract-, or genitourinary tract-origin). Information regarding companion animals (animal species, sex, age, clinical specimen, date collected, infectious disease, or clinical signs) was retrieved from the request sheets.

2. Determination of species identity and antimicrobial susceptibility

Several isolates were identified as SDSE on the basis of their biochemical properties determined by using either the API-20 Strep or Vitek2 system with the GP ID Card (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan) after isolating the strains at three

different institutions. These institutions sent all the isolates, which were identified as SDSE, to one laboratory (Kitasato Institute for Life Sciences) for further genetic analyses. Accurate identification was based on the sequencing results of the PCR-amplified 16S rRNA gene. SDSE identification was accepted if the isolate sequence yielded only one identification option with $\geq 98.7\%$ similarity to the 16S rRNA sequence of SDSE type strain NCFB 1356(T). After determining species identity, we confirmed 55 strains (52 from humans and three from animals) as SDSE; they were derived from 25 sterile and 30 non-sterile samples.

The minimum inhibitory concentrations (MICs) of various antimicrobial agents, including classes of β -lactam, tetracycline, macrolide/lincosamide, fluoroquinolone, and others, against β -hemolytic streptococci were examined by using the broth microdilution method according to the Clinical and Laboratory Standards Institute guidelines with the same ATCC strain (Group G strain D166B, ATCC 12394) as the control [16].

3. Molecular epidemiological analyses

We performed *emm* genotyping, multilocus sequence typing (MLST), and amplification/sequencing of *sicG* at our laboratory (Kitasato Institute for Life Sciences). Briefly, *emm* typing was based on the Centers for Disease Control and Prevention database (<http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>) [9]. The MLST procedure was performed by sequencing seven housekeeping genes (*gki*, *gtr*, *murl*, *mutS*, *recP*, *xpt*, and *atoB*) according to the pubMLST website for *S. dysgalactiae* (<http://pubmlst.org/sdysgalactiae/>). The STs were grouped into clonal complexes (CCs), in which the connected STs were single locus variants differing by only one housekeeping gene [17]. *sicG* was PCR amplified by using two external and internal specific-primer sets, and amplified DNA was sequenced with the external forward primer, as previously described [13]. Furthermore, random amplified polymorphic DNA (RAPD) was analyzed for the *sicG* isolates as well as for the ATCC control strain, by using three different primers (H2, P5, and P6), to clarify the genetic relationship (subclonality) among the *sicG*-possessing strains [18].

The presence of antimicrobial resistance genes, including *tet*(M), *tet*(O), *tet*(K), *tet*(L), and *tet*(S) (tetracycline-resistance determinants) and *erm*(A), *erm*(B), and *mef*(A) (macrolide/lincosamide-resistance determinants), was confirmed by using PCR, as previously described [19, 20]. Additionally, we confirmed the correct resistance determinant sequences of several isolates that were positive for the target genes.

The study protocol was examined and approved by the human ethics committee of the three institutions as well as the ethics

committee of the Sanritsu Zelkova Veterinary Laboratory before starting the investigation.

4. Statistical analysis

Statistical analysis was performed by using Fisher's exact probability test (two-sided) to assess the relationships between *sicG* detection and the molecular epidemiological data or the development of invasive infections. A *P* value of <0.05 indicated statistical significance.

RESULTS

1. Molecular characteristics and antimicrobial susceptibility of the collected SDSE strains

The relationship between *emm* genotype and CC number among the collected SDSE strains ($n=55$) is shown in Table 1. We identified 16 different *emm* types (most prevalent *stG6792*, $n=18$, 32.7%) and twenty different STs (most prevalent ST17, $n=14$, 25.5%). These STs were grouped into five CCs (CC17,

Table 1. Relationship between *emm* type and clonal complex number among *Streptococcus dysgalactiae* subsp. *equisimilis* strains collected

<i>emm</i> type	Clonal complex (CC) number						Total N
	CC17	CC15	CC29	CC8	CC25	Singletons	
<i>stG6792</i>	18*						18
<i>stG485</i>			6			2 (ST37/ST278 [†])	8
<i>stG245</i>		5					5
<i>stC36</i>	2			4			6
<i>stG10</i>		4					4
<i>stG652</i>					2		2
<i>stG480</i>	1			1			2
<i>stG2078</i>	2						2
<i>stC74a</i>			1				1
<i>stG166b</i>					1		1
<i>stG653</i>	1						1
<i>stG840</i>						1 (ST26)	1
<i>stC1929</i>						1 (ST294*)	1
<i>stG4222</i>					1		1
<i>stC9431</i>						1 (ST14*)	1
<i>stG62647</i>						1 (ST20)	1
Total N	24	9	7	5	4	6	55

*There were *Streptococcus dysgalactiae* subsp. *equisimilis* isolates ($n=3$) from companion animals, and the ST294 was a novel one; [†]ST37 and ST278 were double locus variant.

CC15, CC29, CC8, and CC25), an ST37/ST278 group, and singletons (ST14, ST20, ST26, and ST294). There were three SDSE isolates from dogs ($n=2$, *stG6792*/ST17 and *stC9431*/ST14) and a cat ($n=1$, *stC1929*/ST294); the cat-origin ST294 was a novel strain.

The antimicrobial susceptibility test showed that 18 strains are resistant to either macrolide/lincosamide or tetracycline, as well as fluoroquinolone-resistant isolates ($n=5$). In addition, we found eight different single or combined resistance genotypes (most prevalent *erm*(A); $n=7$, 38.9%).

2. Detection of *sicG*-possessing strains and analysis of the association between the presence of *sicG* and microbiological and clinical features

The SDSE strains with *sicG* features and host information from the three central prefectures (2014–2016), are summarized in Table 2. Carbohydrate group G was detected in 30.9% of the *sicG*-possessing isolates. These isolates originated from 16 hu-

man (blood, $n=10$; open pus, $n=3$; sputum, $n=2$; throat swab, $n=1$) and one canine (open pus) samples. The sequences of all *sicG* amplicons exhibited a single pattern and were closely related to the *sicG* sequence of Japanese strain RE378 (containing *stG6792*, 486-bp, 100% homology, accession no. AP011114.1) and Japanese strain TK01 (containing *stG2078*, 485/486-bp, 99.8% homology, AB508817.1), which were already present in the database for BLAST analysis. Multi-alignments of *sicG* from isolate 6 (dog), isolate 14 (human), and isolate 17 (human), as well as that from strain RE378 and strain TK01, are shown in Fig. 1.

All isolates possessing *sicG* carried CC17, which consisted of ST17 ($n=11$), ST205 ($n=3$), or ST206 ($n=3$). In addition, isolates with *sicG* harbored *stG6792* ($n=14$, 82.4%), *stG2078* ($n=2$), or *stC36* ($n=1$). Two isolates were resistant to fluoroquinolone class antibiotics, and one was resistant to tetracycline owing to the presence of *tet*(M). The *sicG* strains were isolated in Saitama ($n=6$), Chiba ($n=6$), and Tokyo ($n=5$).

Table 2. Features of *Streptococcus dysgalactiae* subsp. *equisimilis* strains with streptococcal inhibitor of complement-mediated cell lysis-like gene and the hosts, Japan (2014–2016)

No. of isolates with <i>sicG</i>	Isolation year	Clinical specimen	<i>emm</i> type (subtype)	Sequence type	Allelic profile	Antimicrobial agent resistance class [antimicrobial resistance gene]	Host (age/gender)	Living area in Japan (prefecture)	Clinical infections
1	2014	Blood	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (85 y/F)	Saitama	Cellulitis
2	2014	Blood	<i>stG6792</i> (.3)	205	4-4-1-2-17-44-2	Tetracycline [<i>tet</i> (M)]	Human (88 y/F)	Saitama	Cellulitis
3	2015	Open pus	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (76 y/M)	Saitama	Decubitus infection
4	2015	Open pus	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (3 m/F)	Saitama	Cellulitis
5	2015	Blood	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (80 y/M)	Saitama	Cellulitis
6	2015	Open pus	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Dog (Unknown/F)	Chiba	Pyoderma
7	2015	Blood	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (90 y/F)	Chiba	Bacteremia
8	2015	Blood	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (86 y/M)	Chiba	Bacteremia
9	2015	Blood	<i>stG6792</i> (.3)	206	4-4-4-2-17-6-2	None	Human (74 y/M)	Chiba	Bacteremia
10	2015	Sputum	<i>stG6792</i> (.3)	205	4-4-1-2-17-44-2	None	Human (60 y/M)	Tokyo	Aspiration pneumonia
11	2015	Blood	<i>stG6792</i> (.3)	17	4-4-1-2-17-6-2	None	Human (93 y/F)	Tokyo	Cellulitis
12	2015	Open pus	<i>stG6792</i> (.3)	206	4-4-4-2-17-6-2	None	Human (74 y/M)	Saitama	Decubitus infection
13	2015	Sputum	<i>stG2078</i> (.0)	17	4-4-1-2-17-6-2	Fluoroquinolone	Human (82 y/F)	Tokyo	Aspiration pneumonia
14	2016	Throat swab	<i>stG2078</i> (.0)	17	4-4-1-2-17-6-2	Fluoroquinolone	Human (72 y/M)	Tokyo	Pharyngitis
15	2016	Blood	<i>stG6792</i> (.3)	205	4-4-1-2-17-44-2	None	Human (84 y/M)	Chiba	Bacteremia
16	2016	Blood	<i>stC36</i> (.7)	17	4-4-1-2-17-6-2	None	Human (82 y/F)	Tokyo	Cellulitis
17	2016	Blood	<i>stG6792</i> (.3)	206	4-4-4-2-17-6-2	None	Human (91 y/M)	Chiba	Bacteremia

All the strains with *sicG* belonged to the carbohydrate group G and clonal complex 17.

Abbreviations: *sicG*, streptococcal inhibitor of complement-mediated cell lysis-like gene; y, years; m, months; M, male; F, female.

Sequence of all *sicG* amplicons was single pattern, and was similar to those of *sicG* in RE378 (AP011114.1, *stG6792*) (full length of *sicG* 486 bp, 100% homology) and TK01 (AB508817.1, *stG2078*) (485/486 bp, 99.8%).

We observed statistically significant relationships between *sicG* detection and the presence of either CC17 or *stG6792* ($P < 0.01$ for each). There was also a significant association between *sicG* presence and the development of skin and soft tissue infections ($P = 0.03$). In contrast, although we encountered invasive ($n = 10$) and noninvasive ($n = 7$) cases due to strains with *sicG*, there was

no significant association between the presence of *sicG* and the development of invasive infections ($P = 0.24$).

3. RAPD analysis of genetic relatedness among *sicG* strains

Images based on RAPD of SDSE isolates using different primers (H2, P5, and P6) are presented in Fig. 2. All isolates with *sicG*

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14      -----TTGTTTTTTTATAATAAGTTCATTAATAAATAATTTATTAAGGAGAGAAGGCTAATGA 57
17      TAATTGTTTTTTTATAATAAGTTCATTAATAAATAATTTATTAAGGAGAGAAGGCTAATGA 60
6       TAATTGTTTTTTTATAATAAGTTCATTAATAAATAATTTATTAAGGAGAGAAGGCTAATGA 60
RE378  TAATTGTTTTTTTATAATAAGTTCATTAATAAATAATTTATTAAGGAGAGAAGGCTAATGA 60
TK01    -----ATGA 4
          ****

14      AGATTAATAATTAGTAAACACTACTATTTACATCCCTGTAGCCGTGGCTCTACTAGGAG 117
17      AGATTAATAATTAGTAAACACTACTATTTACATCCCTGTAGCCGTGGCTCTACTAGGAG 120
6       AGATTAATAATTAGTAAACACTACTATTTACATCCCTGTAGCCGTGGCTCTACTAGGAG 120
RE378  AGATTAATAATTAGTAAACACTACTATTTACATCCCTGTAGCCGTGGCTCTACTAGGAG 120
TK01    AGATTAATAATTAGTAAACACTACTATTTACATCCCTGTAGCCGTGGCTCTACTAGGAG 64
          ****

14      CTACACAACCAAGTTTCAGCATCAGCAGCTGAAGCAAGTAATAGCATTATAATACATATA 177
17      CTACACAACCAAGTTTCAGCATCAGCAGCTGAAGCAAGTAATAGCATTATAATACATATA 180
6       CTACACAACCAAGTTTCAGCATCAGCAGCTGAAGCAAGTAATAGCATTATAATACATATA 180
RE378  CTACACAACCAAGTTTCAGCATCAGCAGCTGAAGCAAGTAATAGCATTATAATACATATA 180
TK01    CTACACAACCAAGTTTCAGCATCAGCAGCTGAAGCAAGTAATAGCATTATAATACATATA 124
          ****

14      GTTCGGGTTATGATTATGATGTATATATAGCGGGTTATAAAGAAGGTTTTTCAGGTGCTC 237
17      GTTCGGGTTATGATTATGATGTATATATAGCGGGTTATAAAGAAGGTTTTTCAGGTGCTC 240
6       GTTCGGGTTATGATTATGATGTATATATAGCGGGTTATAAAGAAGGTTTTTCAGGTGCTC 240
RE378  GTTCGGGTTATGATTATGATGTATATATAGCGGGTTATAAAGAAGGTTTTTCAGGTGCTC 240
TK01    GTTCGGGTTATGATTATGATGTATATATAGCGGGTTATAAAGAAGGTTTTTCAGGTGCTC 184
          ****

14      CTGCTCCAACCTTCAGAAGAGATGGAAGATTGGCCATATGACTACCAATTACCGTATATAG 297
17      CTGCTCCAACCTTCAGAAGAGATGGAAGATTGGCCATATGACTACCAATTACCGTATATAG 300
6       CTGCTCCAACCTTCAGAAGAGATGGAAGATTGGCCATATGACTACCAATTACCGTATATAG 300
RE378  CTGCTCCAACCTTCAGAAGAGATGGAAGATTGGCCATATGACTACCAATTACCGTATATAG 300
TK01    CTGCTCCAACCTTCAGAAGAGATGGAAGATTGGCCATATGACTACCAATTACCGTATATAG 244
          ****

14      ATGGCTATATTAAGGGAAAGAATGATAGAAATACTTCTAAAAGTGAGTCTTCAGAATCGA 357
17      ATGGCTATATTAAGGGAAAGAATGATAGAAATACTTCTAAAAGTGAGTCTTCAGAATCGA 360
6       ATGGCTATATTAAGGGAAAGAATGATAGAAATACTTCTAAAAGTGAGTCTTCAGAATCGA 360
RE378  ATGGCTATATTAAGGGAAAGAATGATAGAAATACTTCTAAAAGTGAGTCTTCAGAATCGA 360
TK01    ATGGCTATATTAAGGGAAAGAATGATAGAAATACTTCTAAAAGTGAGTCTTCAGAATCGA 304
          ****

14      ATATTAGGCAAGGTGAAAACCTTTACTCAACCACCTCAAATCCAGCTCCAAGTTCGCCCTA 417
17      ATATTAGGCAAGGTGAAAACCTTTACTCAACCACCTCAAATCCAGCTCCAAGTTCGCCCTA 420
6       ATATTAGGCAAGGTGAAAACCTTTACTCAACCACCTCAAATCCAGCTCCAAGTTCGCCCTA 420
RE378  ATATTAGGCAAGGTGAAAACCTTTACTCAACCACCTCAAATCCAGCTCCAAGTTCGCCCTA 420
TK01    ATATTAGGCAAGGTGAAAACCTTTACTCAACCACCTCAAATCCAGCTCCAAGTTCGCCCTA 364
          ****

14      GTATCCCTAAAGTTCCTAATAACAAAATGGCCTGATAGGAAAAACGATTTTAGCGAACTTT 477
17      GTATCCCTAAAGTTCCTAATAACAAAATGGCCTGATAGGAAAAACGATTTTAGCGAACTTT 480
6       GTATCCCTAAAGTTCCTAATAACAAAATGGCCTGATAGGAAAAACGATTTTAGCGAACTTT 480
RE378  GTATCCCTAAAGTTCCTAATAACAAAATGGCCTGATAGGAAAAACGATTTTAGCGAACTTT 480
TK01    GTATCCCTAAAGTTCCTAATAACAAAATGGCCTGATAGGAAAAACGATTTTAGCGAACTTT 424
          ****

14      CATTGCGTAAACCACTAAATTAATTCGAGTCTTCCTCTGAATGGGGACAAGCTGTGTATT 537
17      CATTGCGTAAACCACTAAATTAATTCGAGTCTTCCTCTGAATGGGGACAAGCTGTGTATT 540
6       CATTGCGTAAACCACTAAATTAATTCGAGTCTTCCTCTGAATGGGGACAAGCTGTGTATT 540
RE378  CATTGCGTAAACCACTAAATTAATTCGAGTCTTCCTCTGAATGGGGACAAGCTGTGTATT 540
TK01    CATTGCGTAAACCACTAAATTAATTCGAGTCTTCCTCTGAATGGGGACAAGCTGTGTATT 484
          ****

14      AGCAGTT----- 544
17      AGCAGTTGTG 550
6       AGCAGTTGTG 550
RE378  AGCAGTTGTG 550
TK01    AG----- 486
          **
    
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Fig. 1. Multi-alignments of isolate 6 (dog), isolate 14 (human), isolate 17 (human), strain RE378, and strain TK01. These alignments were generated with CLUSTAL W version 2.1 (latest version) (<http://clustalw.ddbj.nig.ac.jp/>).

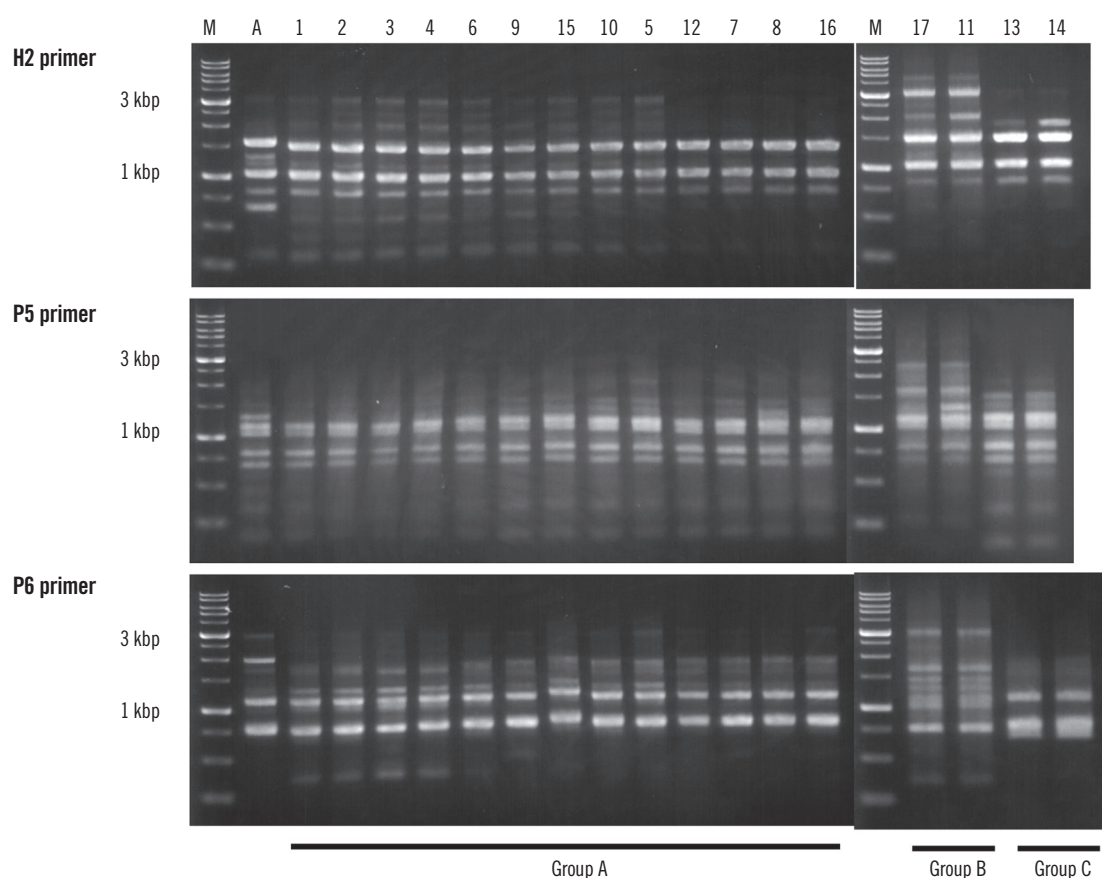


Fig. 2. Random amplified polymorphic DNA analysis of *Streptococcus dysgalactiae* subsp. *equisimilis* isolates with streptococcal inhibitor of complement-mediated cell lysis-like gene (*sicG*), using three different primers: H2, P5, and P6. Abbreviations: M, marker; A, ATCC 12394.

Table 3. Prevalence of *Streptococcus dysgalactiae* subsp. *equisimilis* strains with streptococcal inhibitor of complement-mediated cell lysis-like gene

Isolation country (area)	Study period	N of screened isolates (N of isolates from sterile specimens)	N (%) of <i>sicG</i> -positive isolates	N (%) of <i>sicG</i> -positive isolates from sterile specimens	N (%) of <i>sicG</i> -positive isolates with <i>stG6792</i>	Reference No.
Japan	2000 to 2009	110 (51)	19 (17.3)	11 (57.9)	0	12
Taiwan (central)	2007 to 2011	246 (66)	6 (2.4)	1 (16.7)	3 (50)	21
Norway (western)	2003 to 2009	129 (65)	15 (11.6)	8 (53.3)	1 (6.7)	22
Australia, US, Japan, Portugal, and India	Uncertain*	193 (ND)	30 (15.5)	ND	0	13
Japan (central 3 prefectures)	2014 to 2016	55 (25)	17 (30.9)	10 (58.8)	14 (82.4)	This study

*because of strain donation from multiple countries.

Abbreviations: *sicG*, streptococcal inhibitor of complement-mediated cell lysis-like gene; ND, not described.

were different from the ATCC control strain. The clones harboring both *sicG* and CC17 were divided into three groups (A, B, and C), as demonstrated by RAPD analysis. Group A included 13 strains, whereas groups B and C included only two isolates each, suggesting the spread of the dominant group A sub-clone

into the three central prefectures.

DISCUSSION

Table 3 summarizes our *sicG*-positive SDSE isolate prevalence

data and that from previously published studies [12, 13, 21, 22]. Davies *et al* [23] applied a targeted microarray containing 216 GAS virulence genes to profile the virulence gene repertoires of 58 human-origin SDSE isolates including 46 group G strains (16 from invasive infections) and 12 group C strains (two from invasive infections) in Australia; 9% of the group G isolates carried *sicG*, while no group C isolates harbored *sicG*. Although there are differences in strain source, investigation period, and the number of screened isolates, the detection rate of *sicG*-positive isolates (30.9%) seems to be relatively high in our study, suggesting that it constitutes a clonal type. Interestingly, our findings that *stG6792* (32.7%) was the most prevalent *emm* type, indicating a clonal type, and that a significant proportion of isolates carrying *sicG* (82.4%) also harbored *stG6792*, suggest the clonal combination of *sicG* with *stG6792*. Moreover, the presence of *sicG* was significantly associated with the development of skin and soft tissue infections. In addition, *sicG* strains containing *stG2078* ($n=3$) were consistently associated with severe soft tissue infections [22]. In contrast, there was no significant relationship between *sicG* detection and the development of invasive infections in our survey. As this is a preliminary study conducted over a short period (two years), further investigation of the epidemiological features of additional SDSE strains should be undertaken to clarify the association between the presence of *sicG* and microbiological and clinical features.

Oppegaard *et al* [22] described the sequence diversity of *sicG* (six alleles, *sicG1-6*) among SDSE strains. This diversity was mainly caused by in-frame deletion/insertion mutations involving repeats, along with point mutations leading to non-synonymous substitutions. However, in this study, all *sicG* amplicons ($n=17$) exhibited a single sequence pattern, which was similar to that of *sicG* in strains RE378 (*stG6792*, 100% homology) and TK01 (*stG2078*, 99.8% homology, *sicG4* allele). Likewise, sequencing analysis of other *sicG*-positive isolates ($n=19$) from Japan revealed that their sequence was identical to that in TK01 [12]. In addition, all *sicG* isolates in this study belonged to CC17. To the best of our knowledge this is the first report regarding the association between the presence of *sicG* and CC. The genetic relatedness among *sicG* strains, analyzed by RAPD, suggests the spread of a dominant sub-clone (group A, $n=13$). Therefore, this main sub-clone harboring *sicG* and CC17 might be distributed in the three central prefectures of Japan.

The location of the *sic* gene (915-bp) was previously identified in the chromosome of a GAS M1 strain [10, 24]. This gene is located in the *Mga* regulon, which includes three other virulence genes: *emm1*; *sph*, encoding protein H; and *scpA*, encoding

streptococcal C5a peptidase. The *sic* gene was found in a very limited number of *emm* type GAS strains. However, *sicG* is not located near *emm*. For example, in the genome of RE378, the *sicG* sequence starts at nucleotide 950611, while *emm* (*stG6792*) starts at nucleotide 208440. In the genome of Japanese strain GGS_124 (AP010935.1), the *sicG* sequence starts at nucleotide 917183, whereas the *emm* (*stG480*) sequence starts at nucleotide 204718. Further investigation of the regulatory mechanism and other functions of *sicG* might elucidate the virulent conditions among SDSE isolates from humans and animals.

To the best of our knowledge, this is the first report of a *sicG*-possessing SDSE isolate (CC17 and *stG6792*) from the open pus of a house pet (a dog). Our observations suggest a potential risk for the transfer of some virulent strains between companion animals and human owners and the subsequent clonal spread in a community. Schrieber *et al* [25] documented the findings of an identical SDSE isolate (carbohydrate group C, *stC839.5*, and ST3) using pharyngeal swabs collected from a child and his pet dog in an Aboriginal Australian community. They recommended that the epidemiological investigation of SDSE in hyper-endemic human populations should include strains isolated from animals. Therefore, on the basis of the “One Health” concept [26], an epidemiological survey of SDSE (a zoonotic pathogen) isolated from humans and animals needs to be conducted. Moreover, several SDSE strains are able to transfer between host species and may possess genetic variability and the ability to acquire virulence.

In conclusion, our results suggest the possible spread of three SDSE CC17 subclones with *sicG* in three prefectures in central Japan. Further studies of isolates from diverse geographic areas might be required to monitor the clonal spread of these virulent SDSE strains.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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