

## Short Communication

# Genetic Diversity in *Streptococcus dysgalactiae* subsp. *equisimilis* Isolates from Patients with Invasive and Noninvasive Infections in a Japanese University Hospital (2014–2015)

Tomohiro Fujita<sup>1,2</sup>, Ayaka Horiuchi<sup>3</sup>, Miho Ogawa<sup>4</sup>, Haruno Yoshida<sup>5</sup>,  
Yoneji Hirose<sup>1,2</sup>, Noriyuki Nagano<sup>3</sup>, and Takashi Takahashi<sup>1,5\*</sup>

<sup>1</sup>Department of Infection Control and Prevention; <sup>2</sup>Clinical Laboratory, Kitasato University Medical Center, Saitama; <sup>3</sup>Department of Health and Medical Sciences, Shinshu University Graduate School of Medicine, Nagano; <sup>4</sup>Department of Bacteriology, BML, Inc., Saitama; and <sup>5</sup>Laboratory of Infectious Diseases, Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

**SUMMARY:** *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) isolates with  $\beta$ -hemolysis and carbohydrate groups G or C are increasingly recovered from invasive infections in Japan. The aim of this study was to determine the epidemiological characteristics of SDSE isolates circulating locally among patients with invasive and noninvasive infections. We selected groups G/C  $\beta$ -hemolytic streptococci from a repository at the Clinical Laboratory of Kitasato University Medical Center, from May 2014 through April 2015. Thirteen isolates were identified as SDSE based on the data from API-20 Strep and 16S rRNA sequencing. The samples were from 7 sterile specimens (blood) and 6 non-sterile specimens (pus/sputum/vaginal secretion). Information about the patients with invasive or noninvasive SDSE infections was retrieved from their medical charts. We performed *emm* genotyping, multilocus sequence typing, a dendrogram analysis of the samples using pulsed-field gel electrophoresis (PFGE), and amplifications of the streptococcal inhibitor of a complement-mediated cell lysis-like gene (*sicG*) and antimicrobial resistance determinants. We identified 8 different *emm* genotypes, 8 different sequence types, including 4 novel types, 9 different groups in the PFGE dendrogram, the presence or absence of *sicG*, and 4 different resistance genotypes. Our observations indicate genetic diversity in SDSE isolates from patients with invasive and noninvasive infections in a Japanese university hospital (2014–2015).

*Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) isolates with  $\beta$ -hemolysis and Lancefield carbohydrate groups G or C antigens are increasingly recovered from severe invasive infections (e.g., streptococcal toxic shock syndrome, necrotizing fasciitis, meningitis, infectious endocarditis, sepsis, septic arthritis, and osteomyelitis) worldwide (1). In Japan, 231 invasive SDSE infections were identified, whereas 97 other patients had infections with group A *Streptococcus* (GAS) between August 2006 and July 2007 (2). The SDSE isolation rate from throat swabs was 8-fold higher than the GAS isolation rate among Mumbai school children during 2006–2008 (3). The *emm* gene, which encodes the filamentous surface M protein, is used to type SDSE isolates in epidemiological studies. Of the 229 isolates with determined *emm* types, 55 (24%) had the *stG6792* genotype, which is strongly associated with poor patient outcomes (2). However, few investigators have applied other epidemiological approaches such as mul-

tilocus sequence typing (MLST) or dendrogram analysis by pulsed-field gel electrophoresis (PFGE) to type SDSE strains.

The streptococcal inhibitor of a complement-mediated cell lysis-like gene (*sicG*) encoding a newly discovered extracellular virulence factor, is produced by only a few *emm* types among SDSE strains (4). The DrsG protein encoded by *sicG* has been shown to inhibit the antimicrobial peptide LL-37, which is expressed in sweat as an innate defense system for the skin (5). High rates of antimicrobial resistance to macrolides and tetracycline were also observed among SDSE isolates causing adult invasive and noninvasive infections in France (2006–2010) (6). The aim of this study was to characterize the genetic diversity of SDSE strains circulating among patients with invasive and noninvasive infections in the Kitasato University Medical Center (a 372-bed tertiary-care hospital in Saitama prefecture, Japan) using *emm* typing, MLST, PFGE analysis, detection of *sicG*, and antimicrobial resistance genotyping.

We selected  $\beta$ -hemolytic group G or C streptococci from a repository at the Clinical Laboratory of Kitasato University Medical Center between May 1, 2014 and April 30, 2015 (for 1 year). Thirteen isolates, recovered from 7 sterile specimens (blood) and 6 non-sterile specimens (4 pus/1 sputum/1 vaginal secretion) were selected. The isolates were identified as SDSE based on API-20 Strep testing (SYSMEX bioMérieux, Tokyo,

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\*Corresponding author: Mailing address: Laboratory of Infectious Diseases, Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minatoku, Tokyo 108-8641, Japan. Tel: +81-3-5791-6428, Fax: +81-3-5791-6441, E-mail: taka2si@isci.kitasato-u.ac.jp

Japan) for biochemical properties, and the identification was confirmed by sequencing of the 16S rRNA gene amplified by PCR when there was a low probability that the isolate was SDSE, as determined by API-20 Strep. All of the strains identified as SDSE were stored at  $-70^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  until being processed for further evaluation. We used ATCC 12394 (G group strain D166B) as a quality control (7).

Patient information (e.g., underlying conditions, clinical diagnoses, bacterial culture results of blood sample, therapeutic antimicrobial agents, and outcomes) concerning the presence of invasive or noninvasive diseases was retrieved from medical charts. Invasive infection was defined as the isolation of SDSE from a usually sterile site (2), while a noninvasive infection was defined as that from a non-sterile site. All patients lived within the zone of medical care provided by our hospital. Poor outcomes were defined as death from an invasive infection within 3 weeks of disease onset or disease-associated sequelae following the infection (2). The *emm* genotyping, MLST, and PFGE dendrogram analyses were performed as described previously (2,8,9). Briefly, all *emm* typing was based on the U.S. Centers for Disease Control and Prevention database <<http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>>. MLST was performed by sequencing 7 housekeeping genes (*gki*, *gtr*, *murI*, *mutS*, *recP*, *xpt*, and *atoB*) according to the website for SDSE <<http://pubmlst.org/sdysgalactiae/>>. Sequence types (STs) were grouped into clonal complexes (CCs), in which related STs were single locus variants differing in only one housekeeping gene (8). Profiling by PFGE following DNA digestion with the restriction enzyme *Sma*I was also performed. Clustering on the dendrogram was based on 70% or greater similarity of the digested DNA profiles. We amplified *sicG* and sequenced the amplicons (5). Resistance to antimicrobials was determined by the broth microdilution method, according to the Clinical and Laboratory Standards Institute guidelines for  $\beta$ -hemolytic streptococci (10). The presence of antimicrobial resistance genes, including *erm*(A), *erm*(B), *mef*(A), *tet*(M), *tet*(O), *tet*(K), *tet*(L), and *tet*(S), was confirmed with reference strains (11,12). We used the Fisher's exact probability test (one-sided) for statistical analysis. A *p* value of  $<0.05$  indicated statistical significance.

This study protocol was approved by the ethical committee of Kitasato University Medical Center before the investigation began.

The characteristics of SDSE isolates from invasive and noninvasive infections at our hospital, and the corresponding patient's information are shown in Tables 1 and 2. We identified 8 different *emm* types and 8 different STs, including 4 novel STs (ST155, ST205, ST206, and ST238). ST205 and ST238 contained novel *xpt* allele sequences. The major *emm* types and STs were *stG6792* ( $n = 6$ ) and ST17 ( $n = 5$ ), respectively. The strain population consisted of 4 CCs and a singleton (ST20), and CC17 ( $n = 7$ ) was most prevalent. Based on PFGE dendrogram analysis, the 14 SDSE strains (including ATCC 12394) were classified into 9 different groups, groups of A to I (Fig. 1), and we found 4 clusters (A1-3, B1-2, C1-2, and D1-2) that included multiple strains. There were 5 strains, KM4,

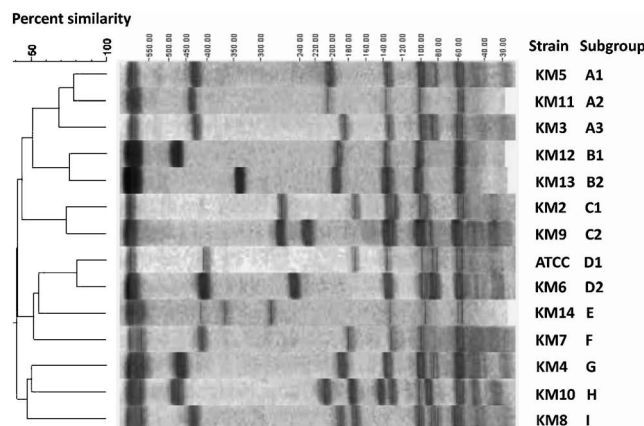


Fig. 1. Pulsed-field gel electrophoresis dendrogram based on the digested DNA profiles of SDSE isolates. The samples were from *Streptococcus dysgalactiae* subsp. *equisimilis* isolates (KM2 to KM14) recovered from inpatients/outpatients at a Japanese university hospital. ATCC 12394 (grouping strain D166B) was the control strain.

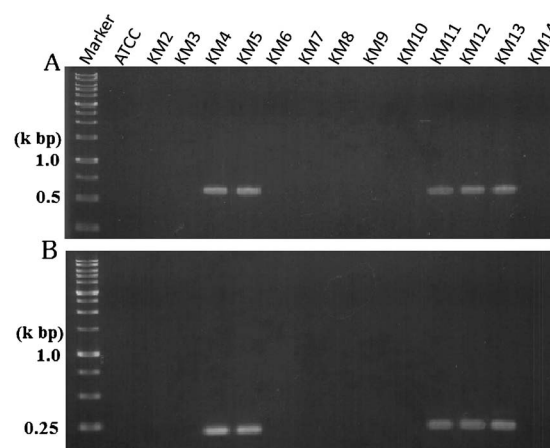


Fig. 2. PCR detection of streptococcal inhibitor of a complement-mediated cell lysis-like gene (*sicG*) in *S. dysgalactiae* subsp. *equisimilis* strains. Representative gels demonstrate amplification of *sicG* from *S. dysgalactiae* subsp. *equisimilis* chromosomal DNA by use of external (A) and internal (B) primer sets. Markers are shown to the left of the gels.

KM5, KM11, KM12, and KM13, that possessed *sicG*, all of which had sequences that were similar to that of *sicG* in the TK01 strain isolated from Japan (Table 1/ Fig. 2). All strains with *sicG* were *stG6792*, and caused invasive or noninvasive skin and soft tissue infections, including cellulitis or decubitus infection (Tables 1 and 2). There were statistically significant associations between possessions of the *sicG* and an *emm* type of *stG6792* ( $p = 0.0047$ ) and onset of skin and soft tissue infections ( $p = 0.0435$ ). Five isolates were resistant to the antimicrobial classes: macrolides, lincomycins, tetracyclines, or fluoroquinolones, and these represented 4 different resistance genotypes, *erm*(B) and *tet*(M), *tet*(M) alone, *erm*(A) alone, and *tet*(O) alone (Table 1).

All patients with invasive disease were elderly and had underlying medical conditions, of which diabetes mellitus and hypertension were most common ( $n = 3$  for each). Strains KM5 and KM3 were from 2 patients with invasive diseases resulting in poor outcomes (death

Table 1. Characteristics of *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) isolates from invasive and noninvasive infections at Kitasato University Medical Center, Japan (2014–2015)

Strain	Isolation date (yr/mon/day)	Clinical specimen	Carbohydrate group	Code No. by API-20 Strep (% probability)	Similarity to 16S rRNA sequence of SDSE type strain (%)	<i>emm</i> type (subtype)	Sequence type (clonal complex No.) <sup>1)</sup>	Allelic profile: <i>glt-gtr-murI- mutS-recP- xpt-atoB</i> <sup>1)</sup>	Subgroup on PFGE dendrogram (% similarity to another subgroup)	PCR detection of <i>sicG</i> <sup>2)</sup>	Antimicrobial agent resistance class (antimicrobial resistance genes)
<b>Invasive</b>											
KM2	2014/6/6	Blood	G	0463015 (98.5)	99.85	<i>stC74a</i> (.0)	29 (29)	3-2-4-2-7-1-3	C1	–	None
KM3	2014/8/29	Blood	G	0463015 (98.5)	99.92	<i>stG653</i> (.0)	17 (17)	4-4-1-2-17-6-2	A3 (A3 to A1, 70.0)	–	macrolide, lincomycin, and tetracycline ( <i>erm</i> (B) & <i>tet</i> (M))
KM4	2014/10/5	Blood	G	0463015 (98.5)	99.92	<i>stG6792</i> (.3)	17 (17)	4-4-1-2-17-6-2	G	+	None
KM5	2014/10/8	Blood	G	0461415 (77.3)	99.92	<i>stG6792</i> (.3)	205* (17)	4-4-1-2-17-44*-2	A1	+	tetracycline ( <i>tet</i> (M))
KM10	2015/1/14	Blood	G	0463415 (93.3)	100	<i>stG652</i> (.0)	25 (25)	3-2-1-5-7-4-3	H	–	macrolide ( <i>erm</i> (A))
KM13	2015/4/6	Blood	G	0463415 (93.3)	99.92	<i>stG6792</i> (.3)	17 (17)	4-4-1-2-17-6-2	B2 (B2 to B1, 75.0)	+	None
KM14	2015/4/17	Blood	G	0463015 (98.5)	100	<i>stG10</i> (.0)	238* (15)	3-3-2-2-9-50*-2	E	–	None
<b>Noninvasive</b>											
KM6	2014/10/27	Vaginal	G	0463415 (93.3)	100	<i>stG62647</i> (.0)	20 (sing)	3-3-2-8-9-6-6	D2 (D2 to D1, 80.0)	–	None
KM7	2014/11/11	Open pus	C	0463415 (93.3)	100	<i>stG4222</i> (.0)	155* (25)	3-18-1-5-7-4-3	F	–	tetracycline ( <i>tet</i> (O))
KM8	2014/11/18	Open pus	G	0463415 (93.3)	99.92	<i>stG6792</i> (.3)	206* (17)	4-4-2-17-6-2	I	–	None
KM9	2015/1/6	Sputum	G	0463015 (98.5)	99.85	<i>stG485</i> (.0)	29 (29)	3-2-4-2-7-1-3	C2 (C2 to C1, 72.7)	–	macrolide and fluoroquino- lone ( <i>erm</i> (A))
KM11	2015/1/30	Open pus	G	0463415 (93.3)	99.92	<i>stG6792</i> (.3)	17 (17)	4-4-1-2-17-6-2	A2 (A2 to A1, 77.8)	+	None
KM12	2015/2/26	Open pus	G	0463415 (93.3)	99.92	<i>stG6792</i> (.3)	17 (17)	4-4-1-2-17-6-2	B1	+	None
ATCC 12394; Grouping strain D166B	2015/2/26	Open pus	G	0433015 (98.9)	100	<i>stG166b</i> (.0)	25 (25)	3-2-1-5-7-4-3	D1	–	None

*sicG*, streptococcal inhibitor of a complement-mediated cell lysis-like gene; +, positive; –, negative; Vaginal, Vaginal secretion; sing, singleton.

<sup>1)</sup>: Asterisk indicates a novel sequence type or allele number.  
<sup>2)</sup>: Sequences of all *sicG* amplicons were similar to that of *sicG* in TK01 strain isolated in Japan. There was statistically significant association of *sicG* possession with *emm* type of *stG6792* ( $p = 0.0047$ ).

or joint contracture). PFGE group A seemed to be related to poor outcomes in these patients. On the other hand, KM5, KM9, and KM11 isolates from 3 patients who died with invasive or noninvasive diseases were typed as *stG6792* with ST205 (CC17) and *sicG*, *stG485* with ST29 (CC29) but not possessing *sicG*, and *stG6792* with both ST17 (CC17) and *sicG*, respectively.

Ahmad et al. (13) assessed the genetic relationships among *emm* types and STs of invasive SDSE strains in the USA. The isolates with prevalent *emm* types belonged to identical or nearly identical STs, indicating concordance between the *emm* type and genetic relatedness. However, several strains with identical or similar STs exhibited multiple unrelated *emm* types, suggestive of recombination event involving the *emm* gene. Lateral gene transfer and recombination between housekeeping genes and the *emm* gene are important mechanisms that lead to genetic variability in SDSE isolates (8). The genetic variability among isolates obtained at the Kitasato University Medical Center was evident.

The most common *emm* type and ST combination was *stG6792* and ST17 ( $n = 4$ : strains; KM4, KM13, KM11, and KM12). This combination accounted for 3 groups (G, B2, A2, and B1) in the PFGE dendrogram (Table 1/Fig. 1). Thus, the PFGE analysis appears to have better discriminatory power than *emm* genotyping and/or MLST for bacterial strains, especially those from our study area. In accordance with our findings, a genetic analysis of invasive ( $n = 21$ ) and noninvasive ( $n = 49$ ) infections in Taiwan identified 51 different PFGE types among GAS isolates lacking particular *emm* types (14).

There is a difference in the use of each typing method (*emm* typing, MLST, and PFGE analysis). Both *emm* typing and MLST are useful as standards recommended for international comparison of strain types, whereas the PFGE method is beneficial in analyzing the genetic relatedness of isolates circulating locally. When there are little correlation between these 3 molecular tests, the results from each method should be interpreted depending on the study purpose. Therefore, we gave priority to the PFGE results. Less correlation between the sequence-based and non-sequence-based techniques (MLST vs. PFGE method) was observed in methicillin-resistant *Staphylococcus aureus* isolates as well as in SDSE strains (15). However, investigators need to be aware of the difficulty of performing the PFGE with the mucoid strains of streptococcus.

Interestingly, we found 5 strains (KM4, KM5, KM11, KM12, and KM13) with both of *sicG* and *stG6792*. Oppegaard et al. (16) recently reported *sicG* sequence diversity among SDSE isolates associated with human infections in western Norway and observed 1 strain with the same combination as found in our study. These data indicate a statistically significant relationship between this special *emm* type and the restricted possession of *sicG*. Moreover, our 5 isolates were significantly associated with skin and soft tissue infections (cellulitis or decubitus infection). Thus, isolates with *sicG* seem to preferentially colonize skin via inhibition of antimicrobial peptide LL-37 activity.

Here, the patients with noninvasive disease ranged from infant to elderly; however, all patients with invasive diseases were elderly with underlying conditions, as

Table 2. Invasive and noninvasive SDSE infections at Kitasato University Medical Center, Japan (2014–2015)

Strain	Inpatient or outpatient (age [yr], sex)	Underlying medical condition <sup>1)</sup>	Infectious disease <sup>2)</sup>	Clinical department	Therapeutic anti-microbial agent	Outcome
<b>Invasive</b>						
KM2	Inpatient (87, W)	Colon cancer with pulmonary metastasis	Meningitis	ED	ampicillin	Alive w/o sequela
KM3	Inpatient (69, W)	Hypertension	Multiple septic arthritis with shock	Orthopedics	penicillin G	Alive with joint contracture
KM4	Inpatient (85, W)	Rheumatoid arthritis, cerebral infarction, and decubitus	Cellulitis	Gastrointestinal medicine	subbactam and ampicillin	Alive w/o sequela
KM5	Inpatient (88, W)	Diabetes mellitus, cerebral infarction, and heart failure	Cellulitis with septic shock	Neurology	cefazolin	Died
KM10	Inpatient (86, W)	Hypertension, hyperlipidemia, and gastroesophageal reflux disease	Vertebral osteomyelitis	Orthopedics	ampicillin	Alive w/o sequela
KM13	Inpatient (80, M)	Diabetes mellitus	Cellulitis	Gastrointestinal medicine	ampicillin	Alive w/o sequela
KM14	Inpatient (70, W)	Diabetes mellitus, hypertension, and post-operation for cervical cancer	Cellulitis	ED	meropenem	Alive w/o sequela
<b>Noninvasive</b>						
KM6	Outpatient (62, W)	None	Vaginitis	Gynecology	None	Alive w/o sequela
KM7	Outpatient (74, W)	Cerebral infarction and cutaneous ulcer	Cellulitis	Dermatology	cefdirinir	Alive w/o sequela
KM8 <sup>3)</sup>	Outpatient (83, W)	Polymyositis with prednisolone treatment	Subcutaneous abscess	Dermatology	cefdirinir	Alive w/o sequela
KM9	Inpatient (94, W)	Bedridden status needing total assistance	Aspiration pneumonia	Connective tissue disorders	subbactam and ampicillin	Died
KM11	Inpatient (76, M)	Malnutrition	Decubitus infection	Nephrology	flomoxef	Died
KM12	Inpatient (3 mon, W)	None	Cellulitis	Pediatrics	cefazolin	Alive w/o sequela

SDSE, *Streptococcus dysgalactiae* subsp. *equisimilis*; W, woman; M, man; ED, Emergency department; w/o, without.

<sup>1)</sup>: No patients received surgical procedures (including debridement).

<sup>2)</sup>: There was statistically significant association of *sicG* possession with onset of skin and soft tissue infections ( $p = 0.0435$ ).

<sup>3)</sup>: Only 1 patient (KM8) received antibiotic of levofloxacin prior to culture.

was found in another study (17). Clinicians examining elderly patients with underlying illnesses who present with a high fever need to perform blood cultures before antibiotics are administered to detect pathogens such as SDSE. Several strains possessing antimicrobial resistance determinants in our study exhibited antimicrobial resistance (e.g. resistance against macrolides and/or fluoroquinolones) in accordance with previous descriptions (2,18). Therefore, antimicrobial resistance should be considered when these alternative drugs are not effective in clinical practice.

In conclusion, our observations reveal genetic diversity (*emm* genotypes, STs, PFGE dendrogram groups, the presence or absence of *sicG*, and antimicrobial resistance genotypes) in SDSE isolates from invasive and noninvasive infections in a university hospital in Japan (2014–2015). We also found significant associations between possession of *sicG* and the *emm* type *stG6792* and onset of skin and soft tissue infections. Further investigations of the epidemiological characteristics of additional SDSE strains should be undertaken to explore the relationship between these strains and patient outcomes.

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**Conflict of interest** None to declare.

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