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Characterization of Group C and G Streptococcal Strains That Cause Streptococcal Toxic Shock Syndrome

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Twelve strains (the largest number ever reported) of group C and G_1 streptococci (GCS and GGS, respectively) that caused streptococcal toxic shock syndrome (STSS) were collected and characterized. Eleven strains were identified as *Streptococcus dysgalactiae* subsp. *equisimilis*, and one strain was identified as *Streptococcus equi* subsp. *zooepidemicus*. We found that it was the first reported case of STSS caused by *S. equi* subsp. *zooepidemicus*. Cluster analysis according to the 16S rRNA gene (rDNA) sequences revealed that the *S. dysgalactiae* strains belonged to clusters I and II, both of which were closely related. The *emm* types and the restriction patterns of chromosomal DNA measured by pulsed-field gel electrophoresis were highly variable in these strains except BL2719 and N1434. The 16S rDNA sequences and other characteristics of these two strains were indistinguishable, suggesting the clonal dissemination of this particular *S. dysgalactiae* strain in Japan. As the involvement of superantigens in the pathogenesis of group A streptococcus-related STSS has been suggested, we tried to detect known streptococcal superantigens in GCS and GGS strains. However, only the *spegg* gene was detected in seven *S. dysgalactiae* strains, with none of the other superantigen genes being detected in any of the strains. However, the *sagA* gene was detected in all of the strains except Tokyo1291. In the present study no apparent factor(s) responsible for the pathogenesis of STSS was identified, although close genetic relationships of GCS and GGS strains involved in this disease were suggested.

A toxic shock-like syndrome due to group A streptococcus (GAS) (*Streptococcus pyogenes*) was reported in the late 1980s (8). Since then the number of patients of this serious infectious disease has been increasing in North America, Europe, and other parts of the world (2, 10, 18, 21, 34). The Working Group on Severe Streptococcal Infections suggested a case definition of streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (52). STSS caused by GAS was characterized as a severe infectious disease which progresses to septic shock and multiple organ failure with a frequent occurrence of necrotizing fasciitis.

Recently, case reports referring to STSS caused by the Lancefield group C streptococci (GCS and G_i and GGS, respectively) have also accumulated (1, 2, 11, 14, 17, 24, 27, 30, 35, 38, 45, 49; H. Watanabe (ed.), Rep. 24th Hyg. Microbe Meet., p. 23–27, 2002 [In Japanese]). However, epidemiological analysis and study for toxin production profiles of the causative organisms have not been fully conducted yet, whereas such analysis and study have been carried out for those organisms associated with GAS-induced STSS. Most clinical isolates of GCS and GGS show beta-hemolysis on sheep blood agar, and some isolates are susceptible to bacitracin. Therefore, these beta-hemolytic GCS and GGS may be misidentified as GAS unless Lancefield serologic testing is performed. Beta-hemolytic GCS include *S. equi* (subspecies *equi* and *zooepidemicus*), *S. dysgalactiae* (subspecies *dysgalactiae* and *equisimilis*), *S. anginosus*, and *S. constellatus*, and beta-hemolytic GGS include *S. dysgalactiae*, *S. canis*, *S. uberis*, *S. intestinalis*, and *S. anginosus*, respectively. Some of these bacteria cause various infections in domestic animals and, sometimes, in humans. Previous studies have demonstrated that none of these beta-hemolytic GCS and GGS secrete any major exotoxins (17, 27, 35, 38, 45, 49). Unlike GAS-induced STSS, underlying conditions have been noted in most patients with STSS related to GCS and GGS. These patients had cardiopulmonary disease, diabetes mellitus, malignancy, and renal or hepatic failure, etc.

In the present study we collected and characterized GCS and GGS strains that caused STSS. We determined the 16S rRNA gene (rDNA) sequences of these strains for identification of the species, since the 16S rDNA is highly conserved within a streptococcal species and can be used as the "gold standard" for identification of the species of bacteria (51). We performed epidemiological analyses with chromosomal pulsed-field gel electrophoresis (PFGE) and *emm* typing of these GCS and GGS strains. We further searched for the genes encoding superantigenic or mitogenic toxins, including a number of novel streptococcal exotoxins that are believed to express superantigenic or mitogenic activities (1, 39, 49), for more understanding of the pathogenesis of this rare, severe infection.

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MATERIALS AND METHODS

Bacterial strains. GGS and GCS strains collected in this study are shown in Table 1. All these strains were recently isolated as causative organisms from STSS patients in Japan. *S. pyogenes* SF370 was provided courtesy of J. J. Ferretti

TABLE 1. Species and *emm* type of GCS and GGS strains used in this study

Strain	Lancefield classification	Species	*emm* type	Reference
BL2719	G	*S. dysgalactiae* subsp. *equisimilis*	*stg2078*	This study
1149	G	*S. dysgalactiae* subsp. *equisimilis*	Novel	17
1201	C	*S. dysgalactiae* subsp. *equisimilis*	*stc839*	17
Fukuoka	G	*S. dysgalactiae* subsp. *equisimilis*	*stg6-1*	27
Tokyo1291	C	*S. equi* subsp. *zooepidemicus*	Not amplified	This study
N1434	G	*S. dysgalactiae* subsp. *equisimilis*	*stg2078*	This study
Hiroshima	G	*S. dysgalactiae* subsp. *equisimilis*	Novel	14
Ichi	G	*S. dysgalactiae* subsp. *equisimilis*	*stc74a*	This study
A-22-84	G	*S. dysgalactiae* subsp. *equisimilis*	*stc36*	This study
A-ka	G	*S. dysgalactiae* subsp. *equisimilis*	*stg480*	This study
A-yo	G	*S. dysgalactiae* subsp. *equisimilis*	*stg485*	This study
A-sa	G	*S. dysgalactiae* subsp. *equisimilis*	*stg11*	45

(13, 44). This strain carried superantigenic genes of *speB*, *speC*, *speF*, *mf-2*, *mf-3*, *speG*, *speH*, *speI*, *speJ*, *smeZ*, and *sagA* and was used as a positive control. *S. pyogenes* 1246 (29) and *S. pyogenes* Ji are clinical isolates harboring *speA*, *speC*, and *speL* and were also used as positive controls to detect these superantigenic genes.

Identification of bacteria. The GGS and GCS strains that caused STSS were identified on the basis of phenotypic characteristics as determined by a rapid ID 32 STREP kit (bioMérieux, Marcy-l'Etoile, France). Phylogenetic analysis of these strains was performed with 16S rDNA sequence. The 16S rDNA was amplified by PCR using a thermal cycler (GeneAmp PCR 9700; Applied Biosystems, Foster City, Calif.) and degenerate primers 27F [AGAGTTTGATC(A/C)TGGCTCAG] and 1492R (GGCTACCTTGTTACGACTT). PCR template DNA was prepared according to the *emm* typing protocol (http://www.cdc.gov/ncidod/biotech/strep/strepindex.html). The PCR for 16S rDNA was carried out by denaturation over 2 min at 94°C, followed by 30 cycles of 60 s at 94°C, 45 s at 55°C, and 90 s at 72°C with a final extension at 72°C for 5 min. PCR products were purified with a Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and were sequenced on a CEQ 2000 DNA analysis system (Beckman Coulter, Fullerton, Calif.) using a Beckman Dye terminator cycle sequencing kit (CEQ DTCS kit) with the primers 27F, 323F (AGACACGGCCCAGACTCCTA), 702F (GTAGCGGTGAAATGCGTAGA), 683R (CTACGCATTTCACCGCTAC), 1238R (TTGTAGCACGTGTGTAGCCC), and 1492R. These kits were used according to the manufacturer's instructions. The search for 16S rDNA sequences of streptococci was performed using Similar Rank in the RDP database (28). The 16S rDNA sequences were aligned using CLUSTAL W software (46). Kimura's two-parameter model was applied for the calculation of evolutionary distance (25), and a phylogenetic tree was constructed by using the neighbor-joining method. Bootstrap analyses of 1,000 replicates were carried out using CLUSTAL W. The classification of *S. dysgalactiae* into two subgroups was based on the recent approval of this classification (47, 48). The 16S rDNA sequences used for depicting a phylogenetic tree include the sequences of *S. dysgalactiae* subsp. *dysgalactiae* ATCC 43078 (GenBank accession no. AB002485) and ATCC 27957 (AB002484); *S. dysgalactiae* V26 (AB002512), A5 (AB002490), L7 (AB002507), A24 (AB002488), A7 (AB002492), L1 (AB002494), V24 (AB002510), L33 (AB002501), and A20 (AB002487); *S. dysgalactiae* subsp. *equisimilis* AC-2074 (AJ314611), AC-2832 (AJ314611), AC-2713 (AJ314609), and NCFB1356 (AB008926); *S. uberis* JCM 5709 (AB023573) and HN1 (AB023576); *S. equi* subsp. *zooepidemicus* ATCC 19615 (AB002516); *S. equi* subsp. *equi* ATCC 33398 (AB002515); *S. pyogenes* ATCC 19613 (Y12924); and *S. pneumoniae* MFF911410 (AB002522).

Typing of M protein gene (*emm*). The M protein types of the GCS and GGS strains were determined according to the M protein gene (*emm*) typing protocol by the sequencing of the *emm* gene (http://www.cdc.gov/ncidod/biotech/strep/strepindex.html).

Susceptibility testing and detection of resistance genes. Antimicrobial susceptibilities were determined by a MicroScan (Dade Behring, Deerfield, Ill.) microdilution method and Etest (AB Biodisk, Solna, Sweden) and were confirmed by an agar dilution method.

Erythromycin resistance genes (*ermB*, *ermTR*, and *mefA*) were searched by PCR (12, 42, 43). For amplification of erythromycin resistance genes, the following primers were used: 5′-GAAAARGTACTCAACCAAATA-3′ (R indicates A or G) and 5′-AGTAACGGTACTTAAATTGTTTAC-3′ for *ermB* (43), 5′-TCAGGAAAAGGACATTTTAC-3′ and 5′-ATACTTTTTGTAGTCCTTCTTT-3′ for *ermTR* (designed on the basis of the homologous region of *ermTR* and *ermA*), and 5′-AGTATCATTAATCACTAGTGC-3′ and 5′-TTCTTCTGGTACTAAAAGTGG-3′ for *mefA* (43).

The tetracycline resistance gene (*tetM*) was detected by PCR (4, 23). The sequences of the primers for amplification of *tetM* were 5′-TTATCAACGGTTTATCAGG-3′ and 5′-CGTATATATGCAAGACG-3′.

PCR for erythromycin resistance genes (*ermB*, *ermTR*, and *mefA*) was carried out by denaturation over 2 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 49°C, and 90 s at 72°C with a final extension at 72°C for 5 min. For *tetM*, the annealing temperature was changed to 50°C.

Detection of superantigenic genes and streptolysin S gene (*sagA*) by PCR. PCR primers were designed to detect superantigenic genes *speA*, *speB*, *speC*, *speF*, *speH*, *speI*, *speJ*, *speL*, *mf-2*, *mf-3*, and *smeZ* found in *S. pyogenes* and a superantigenic gene, *spegg* (GenBank accession no. AJ294849). *spegg* is a homologue of the *S. pyogenes* superantigenic gene *speG* and was found in *S. dysgalactiae* subsp. *equisimilis* (39). PCR template DNA was the same as that for *emm* typing. For amplification of superantigenic genes as well as the *sagA* gene, primers listed in Table 2 were designed according to the indicated references.

PCR for *speA*, *speC*, *spegg*, and *speI* was carried out by denaturation over 2 min at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 51°C, and 90 s at 72°C with a final extension at 72°C for 5 min. For *speB*, *speF*, *mf-2*, and *mf-3*, the annealing temperature was changed to 55°C. For *speH*, *speJ*, *speL*, and *smeZ*, the annealing temperature was changed to 49°C. For *sagA*, the annealing temperature was changed to 50°C.

PFGE. PFGE of restriction enzyme-digested chromosomal DNA was carried out according to the previously described method (20). Briefly, chromosomal DNA was digested overnight with *Sma*I (Takara, Kyoto, Japan), and the digested sample was electrophoresed through 1% agarose gel in 0.5× TBE buffer (0.05 M Tris, 0.05 M boric acid, 1 mM EDTA [pH 8]) by using a Gene Navigator pulsed-field system (Amersham Pharmacia Biotech, Uppsala, Sweden). Electrophoresis was carried out at 200 V for 18 h, with the pulse time ranging from 1 to 20 s. Thereafter, the gel was stained with ethidium bromide, washed with distilled water, and photographed. Lambda DNA PFGE markers (Amersham Pharmacia Biotech) were used as size standards.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study were submitted to the DDBJ nucleotide sequence database, and the accession numbers for each strain were given as follows: BL2719 (AB10483), 1149 (AB104840), 1201 (AB104841), Fukuoka (AB104842), Tokyo1291 (AB104843), N1434 (AB104844), Hiroshima (AB104845), Ichi (AB104846), A-22-84 (AB104847), A-ka (AB104848), A-yo (AB104849), and A-sa (AB104850).

RESULTS

Differentiation of GCS and GGS strains and typing of the M protein gene (*emm*). The species of the GCS and GGS strains isolated from STSS patients were determined with a rapid ID 32 STREP kit (bioMérieux). The PCR-amplified 16S rDNA fragments ranging from positions 27 to 1511 of the 16S rDNA sequence of *Escherichia coli* were directly sequenced. These sequences corresponded to an estimated 93% of the total 16S

TABLE 2. PCR primer sets for detection of superantigenic genes and streptolysin S gene (*sagA*)

Gene	Primer	Primer sequence	Reference
speA	*speA*-F	5′-CAACAAGACCCCGATCCA-3′	50
	speA-R	5′-CTTGGTTGTTAGGTAGAC-3′	
speB	*speB*-F	5′-GGCGATTTCAGAATTGATGGC-3′	7
	speB-R	5′-TAGTGCGTCAAGACGGAAG-3′	
speC	*speC*-F	5′-CCGAAATGTCTTATGAGGCC-3′	7
	speC-R	5′-CCCTTCATTTGGTGAGTC-3′	
speF	*speF*-F	5′-ATGAATCTACTTGGATCAAG-3′	This study
	speF-R	5′-TTATTTCTGAGTAGGTGTAC-3′	
spegg	*spegg*-F	5′-ATGAAAACAAACATTTTGACAATTATC-3′	This study
	spegg-R	5′-CTAGTGCGTTTTTAAGTAGATA-3′	
speH	*speH*-F	5′-AATTCTTATAATACAACCAATAGAC-3′	37
	speH-R	5′-TTAGCTGATTGACACATCTA-3′	
speI	*speI*-F	5′-ATGAGTAGTGTGGGAGTTATTAA-3′	36
	speI-R	5′-TTTAGCATACTCTCTCTGTCAC-3′	
speJ	*speJ*-F	5′-GATAGTGAAAATATTAAAGACG-3′	36
	speJ-R	5′-TTATTTAGTCCAAAGGTAAATATC-3′	
speL	*speL*-F	5′-GTCATATCATGTTGTATGCAA-3′	21
	speL-R	5′-GTTTAAGTGAACATCAAAGTG-3′	
mf-2	*mf2*-F	5′-CTAGGGTACGAACTTATCC-3′	16
	mf2-R	5′-TTAGTTTTTAGGAGTGGCAG-3′	
mf-3	*mf3*-F	5′-GCCAGAAAAATTAGAAATTT-3′	16
	mf3-R	5′-TTAAGCCGCTTCTTCAAACT-3′	
smeZ	*smeZ*-F	5′-TTAGAAGTAGATAATAATTCCCTT-3′	37
	smeZ-R	5′-TTAGGAGTCAATTTCTATATCTAAA-3′	
sagA	*sagA*-F	5′-TTACTTCAAATATTTTAGCTACTAG-3′	This study
	sagA-R	5′-TTATTTACCTGGTGTATAGC-3′	

rDNA primary sequence. These sequences were compared with the published 16S rDNA sequences of the streptococcal reference strains as described in Materials and Methods. According to the 16S rDNA sequences a dendrogram depicting the estimated phylogenetic relationships was constructed by the neighbor-joining clustering method (Fig. 1). Three sequence clusters could be defined in *S. dysgalactiae* subsp. *equisimilis*: cluster I contained strains Ichi, BL2719, N1434, 1149, 1201, and A-yo and had a mean 16S rDNA sequence similarity of 99.84 to 100%; cluster II contained Hiroshima, Fukuoka, A-22-84, A-ka, and A-sa and had a mean 16S rDNA sequence similarity of 99.77 to 100%; and cluster III had a mean 16S rDNA sequence similarity of 99.77 to 100%. The 16S rDNA sequences of strains belonging to clusters I and II were close to each other (99.62 to 99.92%), with 99.39 to 99.84% differences from cluster III. The reference strains belonging to cluster III have not been divided into subspecies based on the new classification (47, 48). We identified strains BL2719, 1149, 1201, Fukuoka, N1434, Hiroshima, Ichi, A-22-84, A-ka, A-yo, and A-sa as *S. dysgalactiae* subsp. *equisimilis* and identified strain Tokyo1291 as *S. equi* subsp. *zooepidemicus* based on the results of the rapid ID 32 STREP assay and of 16S rDNA analysis (Table 1).

The M protein type was determined according to the *emm* typing protocol. As Table 1 shows, the *emm* types of BL2719, 1201, Fukuoka, N1434, Ichi, A-22-84, A-ka, A-yo, and A-sa were *stg2078*, *stc839*, *stg6-1*, *stg2078*, *stc74a*, *stc36*, *stg480*, *stg485*, and *stg11*, respectively. The *emm* types of strains 1149 and Hiroshima were novel types. The *emm* gene of Tokyo1291 was not amplified. These results indicate that no specific M proteins are related to the pathogenesis of STSS caused by GCS and GGS.

Detection of superantigenic genes and the *sagA* gene. In previous reports the involvement of superantigens in the pathogenesis of GAS-related STSS has been strongly suspected (26, 32, 33). We therefore tried to detect known streptococcal superantigens and putative superantigens by PCR with specific primers in the GCS and GGS strains that caused STSS. As shown in Table 3, *spegg* was detected in 1149, 1201, Ichi, A-22-84, A-ka, A-yo, and A-sa, but none of the other superantigenic genes were detected in any strains. The *sagA* gene was detected in all strains except Tokyo1291 (Table 3). It should be noted that a negative reaction in PCR implies simply loss or alteration of the target sequences for the primers and the presence of the entire superantigenic gene may not be excluded, although such a possibility is quite low.

Antimicrobial susceptibility and detection of resistance genes. All strains were sensitive to β-lactam antibiotics such as penicillin G (MIC $\leq$ 0.03 μg/ml). These strains were also sensitive to vancomycin (MIC $\leq$ 0.5 μg/ml), trimethoprim-sulfamethoxazole (MIC $\leq$ 0.5 μg/ml), levofloxacin (MIC $\leq$ 1.0 μg/ml), chloramphenicol (MIC $\leq$ 0.5 μg/ml), and rifampin (MIC $\leq$ 1.0 μg/ml). However, for 6 out of 12 strains resistance to tetracycline was high (MIC = 32 μg/ml) or moderate (MIC = 2 to 8 μg/ml). The tetracycline resistance gene *tetM* was detected in both 1149 and Ichi, for which resistance to tetracycline was high. The strain A-sa was resistant to erythromycin, clarithromycin, and clindamycin (MICs $\geq$ 256 μg/ml) and other strains were susceptible to these antibiotics. We detected the erythromycin resistance gene *ermB* in A-sa.

PFGE. PFGE patterns of all 12 GCS and GGS strains are shown in Fig. 2. The restriction patterns of BL2719 and N1434 are indistinguishable from each other. Note that these two

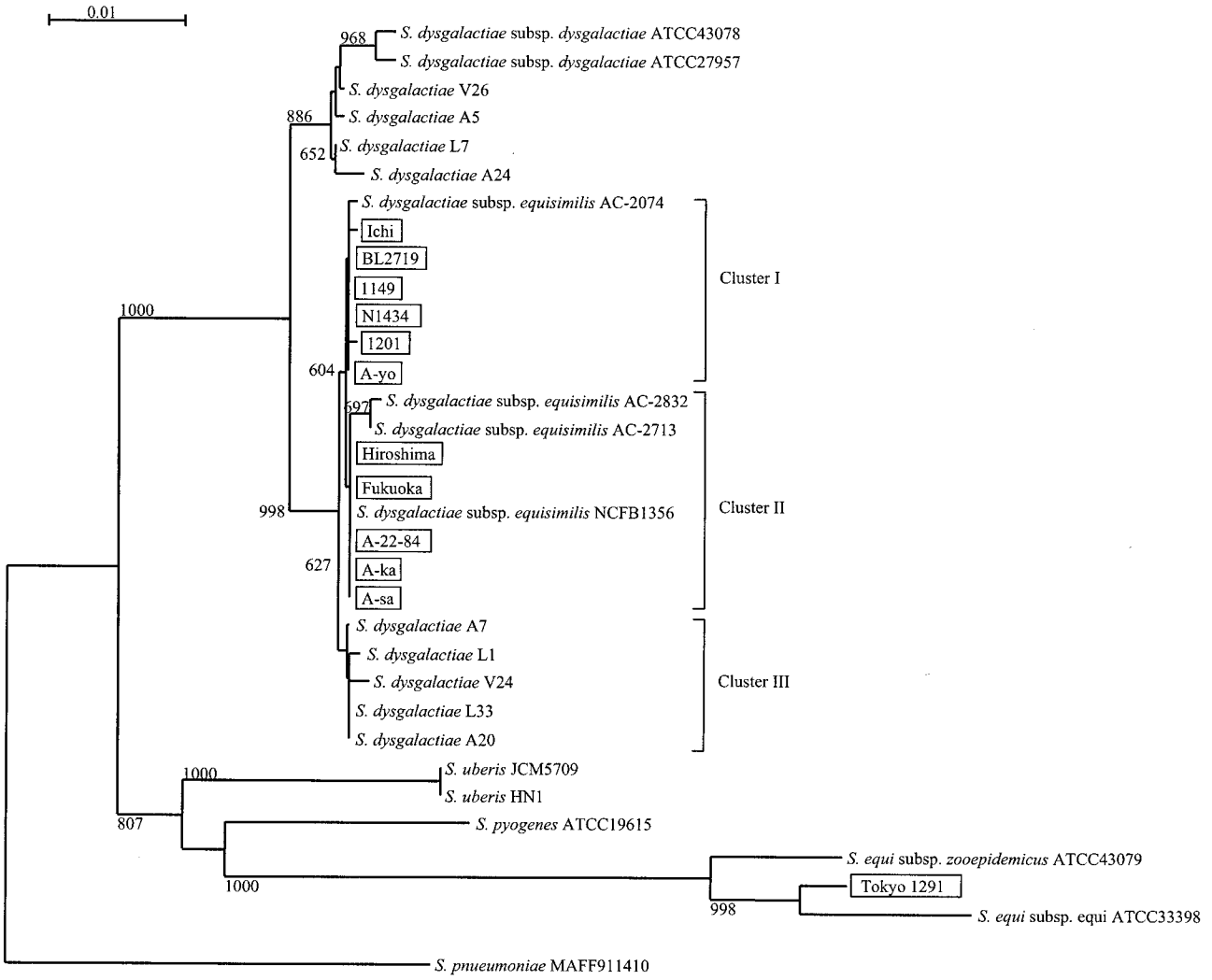

FIG. 1. Phylogenetic tree of GCS and GGS strains isolated from patients with STSS. A phylogenetic tree based on the 16S rDNA sequences was constructed by the neighbor-joining method. Bootstrap analyses of 1,000 replicates were carried out using CLUSTAL W software. Each of the STSS-related strains analyzed in this study is indicated as a square. Sequences of other strains were derived from the GenBank database. Species names of reference strains *S. dysgalactiae* V26, A5, L7, A24, A7, L1, V24, L33, and A20 appeared in the database under the old classification and were not identified into subspecies.

strains were isolated in different places and years. Other strains were genetically diverse.

DISCUSSION

In the past case reports of STSS caused by GCS and GGS (1, 2, 11, 14, 17, 24, 27, 30, 35, 38, 45, 49; H. Watanabe, Rep. 24th Hyg. Microbe Meet.), the causative bacteria in six cases were identified as *S. dysgalactiae* subsp. *equisimilis* (2, 27, 38) and *S. equisimilis* (24, 45), which was renamed as *S. dysgalactiae* subsp. *equisimilis* according to the latest classification (47, 48), while other reports did not identify the species of the causative bacteria. To the best of our knowledge, in the present study we report the first case of STSS caused by *S. equi* subsp. *zooepidemicus*. Clinical information is as follows. A 64-year-old male patient visited an emergency room on foot with lumbago, and 2 h later he lost consciousness and died. Autopsy of the patient indicated necrotic fasciitis of the left arm and underlying cirrhosis. The organism was isolated from the blood culture and the left arm. Strains of *S. dysgalactiae* subsp. *equisimilis* belong to Lancefield group C or G, whereas strains of *S. equi* subsp. *zooepidemicus* belongs to only group C. While *S. dysgalactiae* subsp. *equisimilis* colonizes and causes various infections in humans (51, 53), *S. equi* subsp. *zooepidemicus* is a common pathogen in domestic animals and causes various infections in animals. Therefore, most cases of human infection can be traced to an animal source (5). As our present study and earlier reports have indicated, these organisms secrete only a few superantigenic exotoxins or unknown exotoxins (1, 17, 27, 35, 38, 45, 49). Although clinical manifestations of STSS related to GCS and GGS are the same as those caused by GAS, the involvement of major superantigens such as SpeA, SpeB, and SpeC secreted from GAS are excluded. It has been proposed that streptococcal superantigens, especially pyrogenic exotox-

TABLE 3. Detection of superantigenic genes and streptolysin S gene (*sagA*) by PCR

Strain	Detection of:												
	speA	*speB*	*speC*	*speF*	*spegg*	*speH*	*speI*	*speJ*	*speL*	*mf-2*	*mf-3*	*smeZ*	*sagA*
BL2719	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Positive
1149	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
1201	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
Fukuoka	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Positive
Tokyo1291	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N1434	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Positive
Hiroshima	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Positive
Ichi	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
A-22-84	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
A-ka	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
A-yo	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
A-sa	ND	ND	ND	ND	Positive	ND	ND	ND	ND	ND	ND	ND	Positive
Positive controls													
S. pyogenes SF370	ND	Positive	Positive	Positive	Positive	Positive	Positive	Positive	ND	Positive	Positive	Positive	Positive
S. pyogenes 1246	Positive	Positive	Positive	Positive	Positive	ND	ND	Positive	ND	Positive	Positive	Positive	Positive
S. pyogenes Ji	Positive	Positive	ND	Positive	Positive	ND	ND	ND	Positive	ND	ND	Positive	Positive

[a] ND, not detected.

ins, are potentially responsible for at least some of the manifestations of STSS caused by GAS through the activation of Th1 cells and the liberation of large amounts of interleukins as well as inflammatory cytokines, such as tumor necrosis factor (9). Therefore, unidentified superantigenic exotoxins or exoenzymes may play a role in the development of STSS caused by GCS and GGS. Indeed, it has been reported that some GAS strains that caused STSS lacked one or more major superantigens (2, 18, 21).

Streptolysin S encoded by *sagA* was considered to be a contributing factor in the pathogenesis of streptococcal necrotizing soft tissue infection (19). The *sagA* gene was detected in all

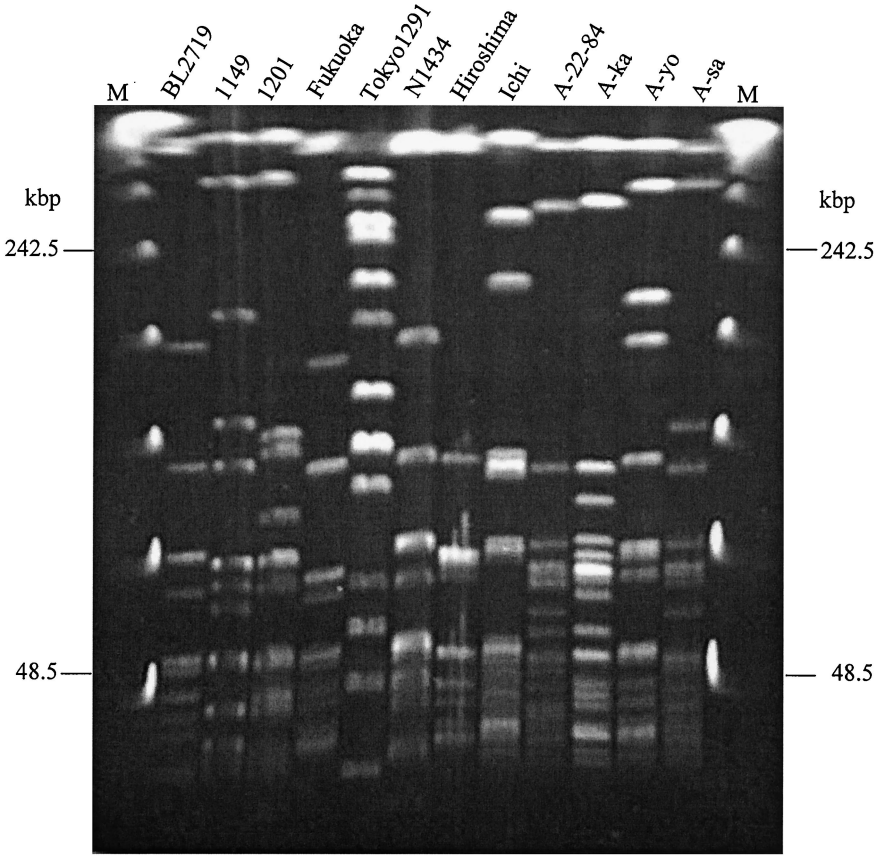

FIG. 2. PFGE separation of *Sma*I-restricted fragments of the chromosomal DNA of the GCS and GGS strains.

of the investigated GCS and GGS strains except Tokyo1291. However, the patient infected with Tokyo1291 showed necrotizing fasciitis, suggesting that streptolysin S may not be essential for the development of streptococcal necrotizing soft tissue infection, or in this case, underlying host factors might have an important role in the pathogenesis.

M protein is known to be the major virulence factor of GAS that inhibits the activation of the alternative complement pathway (9) and impedes phagocytosis by polymorphonuclear leukocytes (9). In epidemiological studies of STSS caused by GAS, M1 and M3 strains were most commonly isolated (2, 22, 34; H. Watanabe, Rep. 23rd Hyg. Microbe Meet.). However, other M serotypes of GAS have infrequently also been isolated from STSS patients. Therefore, the role of specific M proteins in the pathogenesis of STSS is still unknown. Since GCS and GGS have been shown to express M proteins that show high sequence homology to those of GAS (3, 15, 41), M proteins of GCS and GGS may also have antiphagocytic activity. However, whether or not GCS and GGS strains of specific *emm* (M) types are involved in the pathogenesis of STSS remains unknown, since only a few epidemiological studies on *emm* types of GCS and GGS that caused STSS have been performed (H. Watanabe, Rep. 23rd Hyg. Microbe Meet.). We found that the M types of GCS and GGS strains isolated from STSS patients were highly variable, suggesting that no specific types of M proteins were responsible for the pathogenesis of STSS caused by GCS and GGS. Interestingly, M type *stg2078* was identified in two strains that were isolated in different places and on different occasions. Moreover, the PFGE patterns and the sequences of 16S rDNA of these strains were indistinguishable from each other. We therefore suspect the clonal dissemination of this particular *S. dysgalactiae* subsp. *equisimilis* strain in Japan. Additionally, we add two new *emm* types found in this study to the M protein group of *S. dysgalactiae*.

Unlike those with GAS-induced STSS, most patients with STSS caused by GCS and GGS have been noted to have serious underlying diseases. Patients with STSS in this study also had various underlying diseases such as malignancy, chronic renal or hepatic failure, diabetes mellitus, and collagen diseases. Most of these patients were given antibiotic therapy with penicillins and/or other antibiotics. As the present study indicated, all STSS strains were penicillin-sensitive. However, six strains were resistant to tetracycline and one strain was resistant to erythromycin. Among six strains that were resistant to tetracycline, strains 1149 and Ichi harbored *tetM*. Strain A-sa, which was resistant to erythromycin, harbored *ermB*. These sensitivity patterns were similar to those of other reports (6, 31) and GAS strains (6, 31, 40).

A nationwide surveillance for GAS-induced STSS started in Japan in 1999 by the enforcement of a new infection control law. However, cases of GCS and GGS-induced STSS were excluded in that surveillance. Because it seems likely that the frequency of STSS caused by GCS and GGS has increased recently, a surveillance study must be required for more understanding of the disease. In the present study we analyzed a larger number of STSS-causing GCS and GGS strains than had been analyzed in previous reports. Although we found no apparent STSS-related pathogenic factors in these strains, most of the strains belonged to the specific groups of *S. dysgalactiae* subsp. *equisimilis*. Epidemiological studies for more GCS and GGS strains related to STSS are expected to be helpful for the identification of the common pathogenic factors in this disease.

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