

Short Communication

Surveillance of Group B Streptococcal Toxic Shock-Like Syndrome in Nonpregnant Adults and Characterization of the Strains in Japan

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SUMMARY: Nine group B streptococci (GBS) strains were isolated from five toxic shock-like syndrome cases of nonpregnant adults in Japan from 2001 to 2005. All of them were identified as *Streptococcus agalactiae*. The serotypes of these strains were Ib, III, V, and VII. Pulsed-field gel electrophoresis revealed that the patterns of the strains isolated from the different patients were variable. Antimicrobial susceptibility tests showed that all of the strains were susceptible to penicillin G, ampicillin, cefotaxime, clindamycin, and telithromycin. One strain showed intermediate resistance to erythromycin.

Streptococcus agalactiae (group B *Streptococcus* [GBS]) is a Gram-positive coccus that is classified in Lancefield's group B by its cell wall polysaccharide antigens. The bacterium is found in the gastrointestinal tract and vagina of asymptomatic humans (1,2). It mainly infects infants during their birth delivery from mothers who had been colonized with GBS in the birth canal (3,4). GBS is also associated with post-partum infections that may lead to bacteremia and endocarditis in the mother (5). It causes a broad range of disease manifestations from mild skin and soft tissue infections to pneumonia, septicemia, and meningitis in nonpregnant adults, especially in older persons or individuals with underlying chronic illnesses (6,7). The incidence rate of GBS-related invasive disease in nonpregnant adults has been reported to be 10% of that in pregnant women (5,8). Streptococcal toxic shock-like syndrome (STSS) is characterized as a severe *Streptococcus*-related infectious disease which progresses to septic shock and multiple organ failure (9,10). It is mainly due to group A *Streptococcus* (GAS) (*S. pyogenes*). Surveillance investigations of streptococcal infections in Denmark revealed that GBS-related STSS cases were 9% of all cases caused by *Streptococcus* (11). In this study, we describe the clinical features of five GBS-related STSS cases in nonpregnant adults, and we also offer a bacteriological analysis of the GBS strains.

The activity of the Working Group for Streptococci in Japan is based on a network between the National Institute of Infectious Diseases (NIID) and the Prefectural Public Health Institute (PHI); seven branch offices are located in the PHI of Fukushima, Kanagawa, Tokyo, Toyama, Osaka,

Yamaguchi, and Oita. Information on streptococcal infections and clinical isolates is sent to the PHI and NIID from hospitals in Japan. For this study, adults were defined as anyone over 20 years old. The criteria for STSS in streptococcal infections were based in principle on those caused by GAS (9,10); in summary, isolation of *Streptococcus* from sterile body sites, appearance of shock, and two or more of the symptoms (other than shock) that are shown in Table 1. For identification of streptococcal strains, API 20 Strep Kit was used (bioMérieux, Marcy l'Etoile, France). The Lancefield's grouping and serotypes of the strains were determined by using Slidex Strepto-kit (bioMérieux) and a latex agglutination method (Denka Seiken, Tokyo, Japan), which allowed specific detection of polysaccharide antigens.

The presence of the GBS virulence genes, *cylE* (12,13) and *scpB* (14,15), in the GBS strains was investigated by PCR analysis with specific primers. Primers were designed as follows: *cylE* forward, 5'-AAGAGGAGACAGAAATTAGG-3', and reverse, 5'-GGGAGGTAATAATGGAATTG-3'; and *scpB* forward, 5'-CCATTTGATAAACTTGCC-3', and reverse, 5'-ATTAATCACCTTAGCTCCC-3'. The presence in the GBS strains of the streptococcal pyrogenic exotoxin (*spe*) genes (*speA*, *speB*, *speC*, and *speF*) and their homologous genes (*speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, and *speM*) was also investigated by PCR analysis. PCR primers were designed to detect the *spe* genes found in *S. pyogenes*, and reactions were performed as described by Ikebe et al. (16) with Ex Taq polymerase (Takara, Otsu, Japan).

Molecular typing of the GBS strains was analyzed by pulsed-field gel electrophoresis (PFGE), which was performed according to a method as described previously (16). Electrophoresis was carried out at 6 V/cm for 19.5 h, with the pulse time ranging from 2.2 to 54.2 s.

The antimicrobial susceptibility of the strains to eight drugs was analyzed by the broth microdilution method

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according to the Clinical and Laboratory Standards Institute (CLSI) (17,18). The drugs were penicillin G, ampicillin, cefotaxime, ciprofloxacin, erythromycin, clindamycin, telithromycin, and linezolid. The bacterial suspension was added into Dry Plates (Eiken Chemistry, Tokyo, Japan) and incubated at 35-37°C for 22-24 h in ambient air. The breakpoints of resistance to ciprofloxacin and telithromycin were used as described by Ikebe et al. (19).

From 2001 to 2005, five cases of GBS-related STSS in nonpregnant adults were reported to NIID. Symptoms presented in the five cases are listed in Table 1. All five patients showed septic shock and disseminated intravascular coagulopathy but no erythematous rash. Their age ranged from 55 to 74 years; the median was 59. Three patients (C1, C2, and C3) had underlying diseases: cancer, liver cirrhosis, and diabetes (Table 1). The other underlying diseases that had been described by Shimizu et al. (10) were not present in these cases. The mortality rate for the five cases was 40%. A total of nine GBS strains were isolated: six from blood and three from soft tissue, tissue exudate, and granulation (Table 2). Four (NIH227-1, NIH227-2, NIH227-3, and NIH227-4) and two (NIH306-1 and NIH306-2) were isolated at different stages of infection and/or from different specimens of the patients of cases C2 and C4, respectively (Table 2). All of the isolated GBS strains were identified as *S. agalactiae*. Serotypes of these strains were Ib, III, V, and VII (Table 2).

Characteristics of STSS caused by GAS and GBS in our sample of nonpregnant adults in Japan are compared in Table 3. From July 2001 to July 2005, 86 GAS-related STSS cases in nonpregnant adults were reported to us. Cases caused by GBS were only 5.8% of the number caused by GAS. The age range of the GBS-related cases was 55-74, whereas that of the GAS-related ones was 22-90. The mortality rates in these

cases caused by GAS and GBS were 46.5 and 40%, respectively. Appearance rates of the involved symptoms were different between the GAS-related and GBS-related cases, but we are unable to specify a significant difference between them because the number of GBS-related cases was too small.

All of the nine strains possessed the *cylE* and *scpB* virulence genes (Table 2). The *cylE* gene encodes a surface-associated toxin, β -hemolysin/cytolysin (β -H/C; 20), and is responsible for pore-formation in a variety of eukaryotic cell membranes (12,13). The *scpB* gene encodes streptococcal C5a peptidase (ScpB). ScpB is a multifunctional surface-bound protease that specifically inactivates the human phagocyte chemotaxin C5a (14,15). The *cylE* and *scpB* genes, which are involved in the virulence of the bacteria (20-23), are detected in all of the reported and tested GBS including those isolated from non-STSS cases. An association between the presence of the two molecules and the emergence of STSS remains unclear.

The presence of the *spe* genes and their homologous genes, which had been identified in GAS, were investigated in the nine GBS strains because the *spe* genes have been reported to be involved in the pathogenesis of GAS-related STSS (24) and also because the *speA* and *speC* genes were located in temperate phages (25,26) and could transfer to nontoxigenic strains by lysogenic conversion (27). No PCR products corresponding to the *spe* genes and their homologous genes were detected in the GBS strains (Table 2). This result suggests that the *spe* gene family is not associated with the development of STSS in GBS-related cases.

*Sma*I-digested PFGE profiles of the GBS strains were investigated. PFGE patterns of these that were isolated from the same patients (NIH227-1, NIH227-2, NIH227-3, and NIH227-4; NIH306-1 and NIH306-2) were indistinguishable,

Table 1. Clinical features of GBS infections

Case no.	Year/month	Age	Sex	Symptoms ¹⁾								Underlying conditions ¹⁾			Prognosis ²⁾
				Shock	DIC	Consciousness disorder	Liver involvement	Renal impairment	ARDS	Soft-tissue necrosis	Erythematous rash	Cancer	Liver cirrhosis	Diabetes	
C1	2001/7	74	F	+	+	-	-	+	-	+	-	+	+	-	R
C2	2003/2	69	F	+	+	+	+	+	-	+	-	-	+	-	D
C3	2003/5	59	F	+	+	+	+	-	-	+	-	-	-	+	R
C4	2005/5	55	M	+	+	+	+	+	+	-	-	-	-	-	D
C5	2005/7	58	M	+	+	-	+	+	+	-	-	-	-	-	R

¹⁾: +, have symptom; -, no symptom.

²⁾: D, death; R, recovery.

DIC, disseminated intravascular coagulopathy; ARDS, adult respiratory distress syndrome.

Table 2. Characteristics of GBS isolates

Case no.	Strain	Specimen	Serotype	Detection of ¹⁾												
				<i>cylE</i>	<i>scpB</i>	<i>speA</i>	<i>speB</i>	<i>speC</i>	<i>speF</i>	<i>speG</i>	<i>speH</i>	<i>speI</i>	<i>speJ</i>	<i>speK</i>	<i>speL</i>	<i>speM</i>
C1	NIH173	blood	Ib	+	+	-	-	-	-	-	-	-	-	-	-	-
C2	NIH227-1	blood	V	+	+	-	-	-	-	-	-	-	-	-	-	-
	NIH227-2	tissue exudates	V	+	+	-	-	-	-	-	-	-	-	-	-	-
	NIH227-3	blood	V	+	+	-	-	-	-	-	-	-	-	-	-	-
	NIH227-4	soft tissue	V	+	+	-	-	-	-	-	-	-	-	-	-	-
C3	NIH239	blood	Ib	+	+	-	-	-	-	-	-	-	-	-	-	-
C4	NIH306-1	blood	VII	+	+	-	-	-	-	-	-	-	-	-	-	-
	NIH306-2	granulation	VII	+	+	-	-	-	-	-	-	-	-	-	-	-
C5	NIH308	blood	III	+	+	-	-	-	-	-	-	-	-	-	-	-

¹⁾: +, present; -, absent.

cylE, β -hemolysin/cytolysin; *scpB*, C5a peptidase; *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, and *speM*, streptococcal pyrogenic exotoxin and the homologous genes.

Table 3. Comparison of the characteristics of STSS cases caused by GAS and GBS in nonpregnant adults

	GAS	GBS
Surveillance period ¹⁾	2001. 7-2005. 7	2001. 7-2005. 7
No. of cases	86	5
Male/Female ²⁾	45/40 ³⁾	2/3
Age		
Average age	60.7	63.0
Age range	22-90	55-74
Median age	64	59
Symptoms		
Shock	100.0%	100.0%
DIC	65.1%	100.0%
Consciousness disorder	33.7%	60.0%
Liver involvement	44.2%	80.0%
Renal impairment	68.6%	80.0%
ARDS	33.7%	40.0%
Soft-tissue necrosis	81.4%	60.0%
Erythematous rash	14.0%	0.0%
Mortality rate	46.5%	40.0%

¹⁾: The period that the STSS infections occurred.

²⁾: Number of patients of males and females.

³⁾: Sex of one patient is unknown.

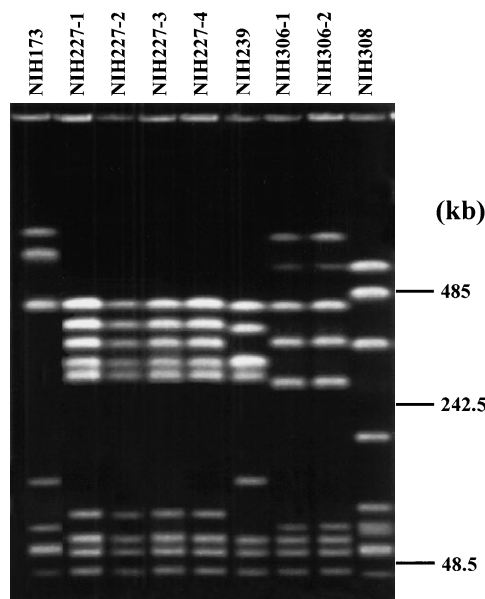


Fig. 1. PFGE of *Sma*I-digested genomic DNAs of GBS strains. The strain name is indicated above each lane and the molecular sizes are shown on the right.

whereas those of strains from different patients were distinct (Fig. 1).

All nine GBS strains were susceptible to penicillin G, ampicillin, cefotaxime, clindamycin, and telithromycin. NIH173 was resistant to ciprofloxacin (MIC $\geq 8 \mu\text{g/ml}$), while the other strains were sensitive. NIH308 was resistant to linezolid (MIC = $4 \mu\text{g/ml}$) and intermediate to erythromycin (MIC = $0.5 \mu\text{g/ml}$) but sensitive to clindamycin (MIC = $0.25 \mu\text{g/ml}$) and telithromycin (MIC $\leq 0.06 \mu\text{g/ml}$); the remaining strains were sensitive to the four antibiotics. Administration of appropriate antibiotics at the early stage of the disease would be the essential treatments for STSS. The combination of penicillin/ampicillin and clindamycin was utilized in three (60%) cases; ampicillin and imipenem were used in one case; and the antibiotics used for the remaining case were unknown.

All of the GBS strains tested in the study were sensitive to penicillin, ampicillin, and clindamycin.

Although GBS diseases in newborns and pregnant women have been studied extensively, investigations of invasive diseases, especially STSS, in nonpregnant adults are still insufficient. In this study, we described five GBS-related STSS cases and investigated the virulent genes, PFGE patterns, and antimicrobial susceptibility of the GBS strains. Because reporting of STSS cases caused by GBS to the Working Group for Streptococci in Japan is not mandatory, a considerable number of severe GBS-related invasive diseases may occur in Japan. Surveillance results in Denmark have revealed that STSS cases are approximately 2.2% of all GBS-related invasive infections (11). Surveillance of infections caused by GBS in adults should be emphasized in Japan.

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