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SHORT REPORT
Surveillance of severe invasive group-G streptococcal infections and molecular typing of the isolates in Japan

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SUMMARY

The number of patients with severe invasive group-G streptococcal (*Streptococcus dysgalactiae* subsp. *equisimilis*) infections has been increasing in Japan. The *emm* genotypes and *Sma*I-digested pulsed-field gel electrophoresis DNA profiles were variable among the strains isolated, suggesting there has not been clonal expansion of a specific subpopulation of strains. However, all strains carried *scpA*, *ska*, *slo* and *sag* genes, some of which may be involved in the pathogenesis of the disease.

Group-G streptococci (GGS) are common members of the normal flora of human skin, pharynx and gastrointestinal tract [1]. GGS can cause pharyngitis, skin and soft tissue infection, septic arthritis, bacteraemia and endocarditis. Since the late 1980s, toxic shock-like syndrome (TSLS) caused by group A *Streptococcus pyogenes* (GAS) has become a serious problem in both developed and developing countries. The characteristic symptoms progress very rapidly and are fulminant from the onset. Patients can develop necrosis of soft tissue, acute kidney failure, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC) and multi-organ failure within 24–72 h, leading to shock and death.

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Recently, it was reported that GGS identified as *S. dysgalactiae* subsp. *equisimilis* also cause streptococcal TSLS [2, 3] but these studies lacked molecular typing and surveillance data. Here, we describe the epidemiological features of severe invasive GGS infections and the character of their isolates in Japan.

The activity of the Working Group for Streptococci in Japan is based on the network between the National Institute of Infectious Diseases (NIID) and prefectural Public Health Institutes (PHIs); six branch offices of the reference centre are located in the PHIs of Yamagata (1995–1997)/Fukushima (1998–2001), Kanagawa, Toyama, Osaka, Yamaguchi and Oita. Information on streptococcal infections and clinical isolates are sent to the PHIs from 3041 cooperative hospitals located throughout Japan. All of them are collected by the NIID. The criteria of

Table 1. *Clinical features of severe invasive group-G streptococcal infections and molecular typing of isolates*

Demographic and clinical information											Strain characterization‡																
			Symptoms*														*speL*	*speL*									
Year /month	Age	Sex	1	2	3	4	5	6	7	D/A†	Strain no.	*emm*	*speG*	*speJ*	*speI*	*speH*	(M3)	(M18)	*speM*	*speA*	*speB*	*speC*	*scpA*	*ska*	*sagA*	*slo*	*sla*
1995/03	69	M	○			○			○	D	25	*stg485*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
1995/10	75	M	○		○	○			○	A	38	*stg840*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
1998/04	64	M	○			○			○	D	183	*stg11*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
1998/09	78	M	○	○	○	○	○		○	A	89	*stg485*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2000/02	90	F	○	○	○	○			○	A	117	*stg6.1*	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2000/04	62	M	○		○			○		D	121	*stg11*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2000/05	78	F	○	○						A	126	*stg840*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2000/04	43	M	○	○	○	○			○	A	140	*stg652*	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2000/10	84	F	○		○				○	D	180	*stg480*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/02	56	F	○	○	○	○			○	A	148	*stc36*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/03	83	F	○						○	A	155	*stg11*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/05	88	M	○	○		○			○	D	159	*stg6.1*	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/05	75	F	○	○	○				○	D	163	*stg6.1*	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/06	80	F			○			○	○	A	174	*stg6792*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/09	63	M		○	○	○	○	○	○	A	181	*stg485*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−
2001/11	79	F	○	○	○	○	○		○	A	182	*stc36*	+	−	−	−	−	−	−	−	−	−	+	+	+	+	−

* 1, Shock; 2, renal impairment; 3, DIC; 4, liver involvement; 5, ARDS; 6, erythematous rash; 7, soft-tissue necrosis.

† D, dead; A, alive.

‡ +, positive; −, negative; *emm*, M-like protein; *speG*, *speJ*, *speI*, *speH*, *speL*(M3), *speL*(M18), *speM*, *speA*, *speB* and *speC*, streptococcal pyrogenic exotoxin genes; *scpA*, C5a peptidase; *ska*, streptokinase; *slo*, streptolysin O; *sagA*, streptolysin S; *sla*, phospholipase A2.

PCR was performed using primers shown in Table 2 and GGS DNA as described previously [7].

Table 2. *Primer sequences used in PCR*

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Reference or accession number
emm	TATT(C/G)GCTTAGAAAATTAA	GCAAGTTCTTCAGCTTGTTT	[7]
speG	AAGAAAATTTCTAATGGAAA	GTAGATATCAAAATGACTAA	AJ294849
speJ	TTTCATGGGTACGGAAGTG	TTATGTATGGAGAATTAGG	AE006504
speI	ACTCTACATATGATCCAACA	TTATAAGAAATTCTCTCTCC	AF438524
speH	CAAATTCTTATAATACAACC	CTAACTTTTATATCCACTTC	AF124500
speL(M3)	GACGAAATTTTGGATAATAG	CTAATCTTTAGAAAAATCTT	AY050245
speL(M18)	TTAATTTTCTTTGTTTGTGT	ATGAGAATTTTTTTACACCA	AE010048
speM	CTAATTTTTAGAAAAATCTTC	TCGCTTGCTCTATACACTAC	AE010048
speA	CTTCAAAATATATATTTTC	TAAATGATTCCCTTCATG	AY049745
speB	GATCAAAACTTTGCTCGTAACG	AGGTTTGATGCCTACAACAGC	M86905
speC	GACTCTAAGAAAGACATTTCG	AGTCCCTTCATTTGGTGAGTC	M35514
scpA	CCATTTGATAAACTTGCC	ATTAATCACCTTAGCTCCC	AE006623
ska	AGTCCAAAATCAAAACCATT	AAATTCTTGGACAGGTTGGG	A20006
sagA	ACTTCAAATATTTTAGCTAC	CTTCCGCTACCACCTTGAG	AY033399
slo	CTTATCCTATTTCATACACC	CTACTTATAAGTAATCGAACC	AB050249
sla	GAAGGGATAAATGATAAAATGG	TTAACATCCTATAGAACCTAC	AY050245

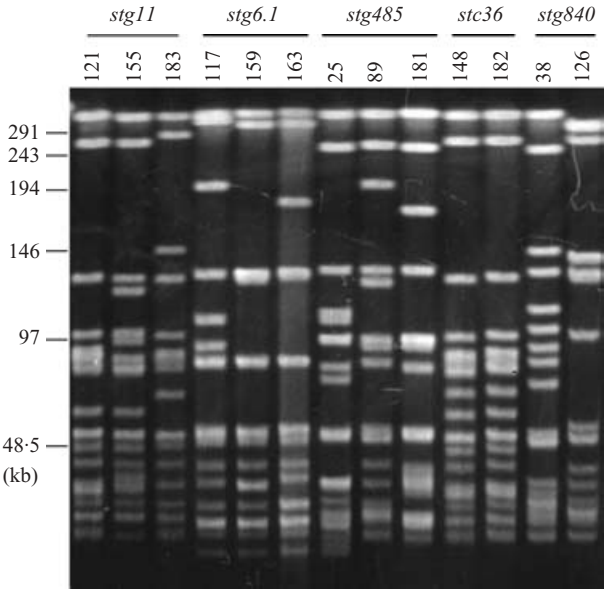

Fig. 1. PFGE of *Sma*I-digested genomic DNA of GGS strains. Strain number is indicated on each lane and the *emm* genotypes are given above.

severe invasive GGS infections were based in principle on those of TSLS [4]; two or more symptoms as shown in Table 1, and isolation of GGS from sterile body sites. From 1995 to 2001, a total of 16 cases of severe invasive infections due to GGS were reported (Table 1) and 12 of them were identified in the most recent period. The median age of the patients was 78 years and ranged from 43 to 90 years, which is older than that (median age 53 years) of TSLS patients ([5] and our unpublished data). Half of the patients were male; 13/16 (81·3%) of the patients had at least one or more underlying diseases: hypertension (6/16, 33·3%), cancer (5/16, 31·3%), cardiac disease (3/16, 18·8%), cirrhosis (3/16, 18·8%), and one patient each had diabetes mellitus, renal disease, tinea pedis, and osteoarthritis. Fourteen patients had clinical shock and varying numbers had renal impairment, DIC, liver involvement, ARDS, a generalized erythematous mascular rash and soft-tissue necrosis (Table 1). All of the GGS isolates were identified as *S. dysgalactiae* subsp. *equisimilis* by Slidex Strepto-kit (bioMérieux Vitek, Tokyo, Japan) and API 20 Strep kits (bioMérieux Vitek). Of the 16 cases, GGS were isolated from blood samples in 11 patients, abscess specimens in 2 patients (12·5%), surgical fluid (2) and joint fluid (1). GGS were not isolated from non-sterile body sites.

GGS express a cell surface M-like protein and at least 23 different forms of the *emm*-like gene (*stg*) [6] have been identified in GGS. We examined the dominant *emm* genotypes among our isolates of severe invasive GGS, by determining the sequence of the *emm* gene as previously described [7]. None of the *emm* genotypes predominated. Eight types of *stg* genes (including one *stc*) were identified: *stg11* (3/16), *stg485* (3/16), *stg6.1* (3/16), *stc36* (2/16), *stg840* (2/16), *stg480* (1/16), *stg652* (1/16) and *stg6792* (1/16). According to the report by Humar et al. [3], the *emm* genotypes of GGS strains from necrotizing infections

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were *stg480* and *stc74c*. Although the *stg480* genotype was also isolated in Japan, GGS with different *emm* genotypes were more prevalent. GGS carrying a particular *emm* genotype do not always cause invasive disease. We isolated two GGS carrying the *emm* gene (*stc36*) found in group C-streptococci, which may be meaningful because the sequences for group C, G and L are associated specifically with *S. dysgalactiae* subsp. *equisimilis*.

We examined *Sma*I-digested pulsed-field gel electrophoresis (PFGE) profiles of the isolates as described previously [8]. Although two *stc36* genotype isolates gave an indistinguishable PFGE pattern, the profiles of almost all the isolates were distinct even among strains of the same *emm* genotype (Fig. 1). Variability of PFGE profiles has also been shown in the isolates of GGS from necrotizing infections [3]. These findings therefore suggest that clonal expansion of GGS was not responsible for the emergence of severe invasive disease.

The presence of specific virulence genes, *scpA*, *ska*, *slo*, *sagA*, *sla*, *speA*, *speB*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speL* (M3), *speL* (M18) and *speM*, was identified by PCR with specific primers (Table 2). All of the isolates carried *scpA*, *ska*, *slo* and *sagA* genes, which may play a role in invasive disease [3]. As the prevalence of *scpA*, *ska*, *slo*, and *sagA* genes among GGS isolates from non-severe cases is unknown, the significance of the genes' involvement in invasive disease is speculative. On the other hand, all strains were negative for *sla*, *speA*, *speB*, *speC*, *speH*, *speI*, *speJ*, *speL* (M3), *speL* (M18) and *speM*. Twelve of the GGS isolates carried the *speG* gene (Table 1).

Our study has shown that the number of patients with severe invasive GGS infections has increased, particularly during 2000 and 2001 in Japan. These strains did not seem to expand clonally as evidenced by the various *emm* genotypes and PFGE profiles. In a previous study, we reported that the number of TSLS cases caused by *S. pyogenes* M3 (*emm3.1*) strains increased rapidly during the period 1993–4 in Japan [5]. These strains had acquired phage DNA (phage NIH1.1), which carried new virulence genes such as *speL* (M3) and *sla* [8, 9]. It is possible that this increase of severe invasive GGS infections by strains with various PFGE profiles is due to the fact GGS has gained new virulence genes that are common among the various *emm* genotype strains by horizontal transfer with bacteriophages or other mobile genetic elements. This clearly requires further investigation.

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