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Increased prevalence of group A streptococcus isolates in streptococcal toxic shock syndrome cases in Japan from 2010 to 2012

T. IKEBE[1]*, K. TOMINAGA[2], T. SHIMA[3], R. OKUNO[4], H. KUBOTA[4], K. OGATA[5], K. CHIBA[6], C. KATSUKAWA[7], H. OHYA[8], Y. TADA[9], N. OKABE[9], H. WATANABE[1], M. OGAWA[1], M. OHNISHI[1] and the Working Group for Beta-haemolytic Streptococci in Japan†

[1] *Department of Bacteriology I, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan;* [2] *Department of Public Health Sciences, Yamaguchi Prefectural Institute of Public Health and Environment, Yamaguchi, Japan;* [3] *Department of Bacteriology, Toyama Institute of Health, Toyama, Japan;* [4] *Department of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan;* [5] *Laboratory of Microbiology, Oita Prefectural Institute of Health and Environment, Oita, Japan;* [6] *Department of Microbiology, Fukushima Institute of Public Health, Fukushima, Japan;* [7] *Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, Osaka, Japan;* [8] *Division of Microbiology, Kanagawa Prefectural Institute of Public Health, Kanagawa, Japan;* [9] *Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan*

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SUMMARY

Streptococcal toxic shock syndrome (STSS) is a severe invasive infection characterized by the sudden onset of shock, multi-organ failure, and high mortality. In Japan, appropriate notification measures based on the Infectious Disease Control law are mandatory for cases of STSS caused by *β*-haemolytic streptococcus. STSS is mainly caused by group A streptococcus (GAS). Although an average of 60–70 cases of GAS-induced STSS are reported annually, 143 cases were recorded in 2011. To determine the reason behind this marked increase, we characterized the *emm* genotype of 249 GAS isolates from STSS patients in Japan from 2010 to 2012 and performed antimicrobial susceptibility testing. The predominant genotype was found to be *emm1*, followed by *emm89*, *emm12*, *emm28*, *emm3*, and *emm90*. These six genotypes constituted more than 90% of the STSS isolates. The number of *emm1*, *emm89*, *emm12*, and *emm28* isolates increased concomitantly with the increase in the total number of STSS cases. In particular, the number of *mefA*-positive *emm1* isolates has escalated since 2011. Thus, the increase in the incidence of STSS can be attributed to an increase in the number of cases associated with specific genotypes.

Key words: Antibiotic resistance, *emm* genotype, group A streptococcus, streptococcal toxic shock syndrome.

INTRODUCTION

Group A streptococcus (GAS) is one of the most common human pathogens and causes a wide array of infections. Many streptococcal virulence factors have been implicated in the pathogenesis of streptococcal infections, including the M protein encoded by the *emm* gene and the Sic protein. The M protein protects GAS from phagocytosis by polymorphonuclear leucocytes [1, 2]. A recent multicentre study analysed 223 *emm*-types of GAS [3]. The Sic protein is incorporated into the complement membrane attack complex and inhibits target cell lysis [4]. Sic is a highly variable

* Author for correspondence: Dr T. Ikebe, Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.
(Email: tdikebe@nih.go.jp)
† Other members of the Working Group are listed in the Appendix.

bacterial protein, and sequencing of the *sic* gene in 500 serotypes of M1 GAS strains recovered from two distinct epidemics in Finland identified 167 Sic variants that emerged rapidly in the course of the epidemics [5].

Since the late 1980s, severe invasive infections, such as streptococcal toxic shock syndrome (STSS), caused by GAS have posed a serious problem in various countries [6–10]. In Japan, STSS is the only invasive GAS infection that is classified as a notifiable disease by the Infectious Disease Control law, and about 60–70 cases are reported annually (T. Ikebe, unpublished results). However, in 2011, a marked increase in STSS (143 cases) was reported in Japan (http://www.nih.go.jp/niid/en/iasren/865-iasr/2505-tpc390.html).

Antibiotic therapy for severe invasive GAS infections entails the administration of a combination of high doses of penicillin and clindamycin. We previously reported that although all GAS isolates were susceptible to penicillin G and ampicillin during 2005–2009, the frequency of clindamycin-resistant strains increased in 2009 [11]. In the present study, we have characterized the *emm*-genotypes and the antimicrobial susceptibility of isolates from STSS during 2010–2012 in Japan.

METHODS

Bacterial isolates

The Infectious Disease Control law classifies STSS as a notifiable disease, and the Working Group for Beta-haemolytic Streptococci collects the causative pathogens from the National Institute of Infectious Diseases (NIID) and the prefectural public health institutes (PHIs) in Japan. Seven branch offices of the reference centre are located in the PHIs of Fukushima, Tokyo, Kanagawa, Toyama, Osaka, Yamaguchi, and Oita. Data on streptococcal infections and clinical isolates are sent to PHIs from hospitals. The number of isolates collected is about 60–70% of the total number of incident cases reported. The diagnostic criteria of GAS-induced STSS were based on the definite cases described by the Working Group on Severe Streptococcal Infections (1993) [12]. A total of 249 GAS isolates from 2010 to 2012 were obtained from sterile body sites of patients with STSS and cultured. Isolates were taken as part of standard patient care.

Ethical statement

This study protocol was approved by the Institutional Individual Ethics Committees for the use of human subjects (the National Institute of Infectious Diseases Ethics Review Board for Human Subjects). The authors assert that all procedures contributing to this work comply with the Helsinki Declaration of 1975, as revised in 2008.

emm- and *sic*-typing

emm gene sequencing was performed as described by Beall *et al*. [13] with modifications described in http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm. *sic* gene sequencing was performed as described by Mejia *et al*. [14]. The *sic* gene sequences obtained in this study have been deposited in the DNA Data Bank of Japan (DDBJ) (http://www.ddbj.nig.ac.jp/index-e.html) database under accession numbers AB911078–AB911102. The 40 new *sic*-types identified in this study were named by adding '-like' to the names of the existing *sic*-types that were the most similar in homology (identified by BLAST search). Cluster analysis was performed using the unweighted pair-group method with mathematical averaging (UPGMA). Alleles of *sic* with >99% similarity were grouped.

Antimicrobial susceptibility tests

The antimicrobial susceptibility of the isolates was measured using the broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [15]. The breakpoints for the resistance of each drug were set according to the recommendations of the CLSI [15]. The double-disk diffusion test was performed using a 15-μg erythromycin disk and a 2-μg clindamycin disk placed 16 mm apart, as described previously [16].

Detection of erythromycin resistance genes

ermA, *ermB*, and *mefA* genes that are responsible for erythromycin resistance were determined by polymerase chain reaction using the published primer sequences [17, 18].

Statistical analyses

Data were compared by Fisher's exact test. Differences were considered significant at $P<0{\cdot}05$.

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Table 1. *Primary focus for streptococcal toxic shock syndrome infections*

Primary focus of infection	No. of cases	%
Skin and soft tissue infection	106	54·4
Abscess	11	5·6
Cellulitis, erysipelas	42	21·5
Necrotizing fasciitis, myositis	48	24·6
Gangrene	4	2·1
Lymphadenitis	1	0·5
Mastitis	1	0·5
Respiratory tract infection	41	21·0
Upper	21	10·8
Lower	20	10·3
Bacteraemia without focus	31	15·9
Arthritis	6	3·1
Gastroenteritis	5	2·6
Meningitis	2	1·0
Peritonitis	1	0·5
Renal failure	1	0·5
Small bowel volvulus	1	0·5
Spondylitis	1	0·5
Total	195	

RESULTS AND DISCUSSION

emm-typing in STSS isolates

The 249 GAS isolates used in this study were collected as follows: 58 isolates in 2010, 89 in 2011, and 102 in 2012. Patients' ages ranged between <1 year and 94 years (interquartile range 49–76); their average age (60·8 years) was less than that of patients with severe invasive group G streptococcal infections during 2002–2008 (average age 66·1 years) [19]. About 55·1% of the study patients were male. Of the 220 cases with described outcomes, the 7-day case-fatality rate was 40·9%. Table 1 summarizes the foci of 195 STSS infections (data unavailable for the other 54 cases) and skin and soft tissues formed the most frequent clinical foci of infection (106/195, 54·4%). This finding is consistent with previously obtained results [20, 21].

A total of 22 *emm* genotypes were identified (Table 2). The ratio of each genotype is presented in Figure 1 and Table 2. The dominant genotype was found to be *emm1* (151/249, 60·6%), followed by *emm89* (30/249, 12·0%), *emm12* (19/249, 7·6%), *emm28* (13/249, 5·2%), *emm3* (6/249, 2·4%), and *emm90* (6/249, 2·4%). These six genotypes constituted more than 90% of the STSS isolates (Fig. 1 and Table 2). The *emm1* genotype was significantly more prevalent during 2010–2012 than in any other period (1996–2000, 53·8%; 2001–2005, 41·6%; 2006–2009, 45·0%; 2010–2012, 60·6%) ($P<0·001$); similarly, the *emm89* genotype was also the most prevalent ($P<0·05$) during this period (1996–2000, 4·3%; 2001–2005, 8·0%; 2006–2009, 5·8%; and 2010–2012, 12·0%).

Compared to 2010, the number of *emm1, emm89, emm12*, and *emm28* isolates increased, while the number of *emm3* isolates decreased (Fig. 1) in 2011–2012, indicating that the increase in the prevalence of these four isolates correlates with the increase in the number of STSS cases.

Among the major *emm* genotypes, the 7-day case-fatality rate by the *emm1*-typed isolates (47·8%) was the highest, higher than the total average (40·9%), and also significantly higher than by any other isolates ($P<0·01$) (Fig. 2). On the other hand, the 7-day case-fatality rates by *emm12, emm28*, and *emm89* were lower than the total 7-day case-fatality rate. A case-fatality rate of 40·9% is relatively high for GAS-induced STSS in Japan compared to other countries [10]. The *emm1* genotype is the most prevalent in STSS, and may lead to the higher rate of 7-day case fatality in Japan.

Several candidate vaccines against GAS infections are in various stages of pre-clinical and clinical development [22]. These 30-valent type-specific, M protein-based vaccines include 93·6% of types that caused STSS in 2010–2012 in Japan (Table 2).

Antimicrobial resistance

We examined the antimicrobial susceptibility of the 249 GAS isolates to six drugs (Table 3). The isolates showed the highest resistance to erythromycin (64·3%), followed by clindamycin (8·4%). All the isolates were susceptible to penicillin G [minimum inhibitory concentration (MIC) range 0·008–0·015 mg/l], ampicillin (0·015–0·06 mg/l), cefotaxime ($\leqslant$0·008 to 0·03 mg/l), and linezolid (1–2 mg/l).

Erythromycin-resistant genotypes

Erythromycin-resistant isolates were obtained annually (2010, 60·3%; 2011, 67·4%; 2012, 63·7%) and of these, isolates carrying the *mefA* gene were prominent and accounted for 86·3% (138/160) of the erythromycin-resistant isolates (Table 4). Almost all of the *mefA*-positive isolates were of the *emm1* genotype. The number of *mefA*-positive *emm1* isolates has increased since 2011, which corresponds to the duration of the sharp increase reported in STSS cases (Table 4).

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Table 2 *Frequency (%) and the number (n) of STSS isolates in each emm genotype reported over time*

	2010		2011		2012		1996–2000		2001–2005		2006–2009		2001–2005	
Genotype	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
emm1	60·3	35	67·4	60	54·9	56	53·8	50	41·6	57	45·0	77	60·6	151
emm3	6·9	4	0	0	2·0	2	1·1	1	9·5	13	9·4	16	2·4	6
emm4	3·5	2	2·3	2	0	0	3·2	3	0·7	1	2·3	4	1·6	4
emm6	0	0	0	0	0	0	3·2	3	2·9	4	0·6	1	0	0
emm9	0	0	1·1	1	1·0	1	0	0	0	0	0·6	1	0·8	2
emm11	1·7	1	0	0	1·0	1	1·1	1	0·7	1	2·9	5	0·8	2
emm12	6·9	4	7·9	7	7·8	8	8·6	8	7·3	10	5·3	9	7·6	19
emm18	0	0	0	0	0	0	1·1	1	0	0	0	0	0	0
emm19	0	0	0	0	1·0	1	0	0	0	0	0	0	0·4	1
emm22	0	0	0	0	1·0	1	5·4	5	2·9	4	1·8	3	0·4	1
emm28	1·7	1	5·6	5	6·9	7	10·8	10	5·1	7	10·5	18	5·2	13
emm31	0	0	0	0	0	0	0	0	0	0	0·6	1	0	0
emm41	0	0	0	0	0	0	0	0	0	0	0·6	1	0	0
emm49	0	0	0	0	0	0	1·1	1	3·6	5	3·5	6	0	0
emm53	0	0	0	0	0	0	0	0	0	0	0·6	1	0	0
emm58	1·7	1	0	0	1·0	1	0	0	3·6	5	0·6	1	0·8	2
emm59	0	0	0	0	0	0	0	0	0	0	0	0	0	0
emm60	0	0	0	0	0	0	0	0	0·7	1	0·6	1	0	0
emm73	0	0	0	0	0	0	0	0	0·7	1	0	0	0	0
emm75	0	0	0	0	1·0	1	1·1	1	2·2	3	2·3	4	0·4	1
emm77	0	0	0	0	0	0	3·2	3	1·5	2	0	0	0	0
emm78	0	0	0	0	0	0	0	0	0·7	1	0	0	0	0
emm79	0	0	0	0	1·0	1	0	0	0	0	0	0	0·4	1
emm81	0	0	0	0	1·0	1	1·1	1	2·9	4	0·6	1	0·4	1
emm87	1·7	1	0	0	0	0	1·1	1	0·7	1	2·9	5	0·4	1
emm89	6·9	4	10·1	9	16·7	17	4·3	4	8·0	11	5·8	10	12·0	30
emm90	1·7	1	2·3	2	3·0	3	0	0	0	0	0	0	2·4	6
emm91	0	0	0	0	0	0	0	0	0·7	1	0·6	1	0	0
emm102	0	0	0	0	1·0	1	0	0	0	0	0	0	0·4	1
emm103	0	0	0	0	0	0	0	0	0·7	1	0	0	0	0
emm106	0	0	1·1	1	0	0	0	0	0	0	0	0	0·4	1
emm112	1·7	1	0	0	0	0	0	0	0·7	1	1·2	2	0·4	1
emm113	5·2	3	0	0	0	0	0	0	0	0	1·8	3	1·2	3
emm118	0	0	1·1	1	0	0	0	0	0	0	0	0	0·4	1
emm177	0	0	0	0	0	0	0	0	0·7	1	0	0	0	0
emm207	0	0	1·1	1	0	0	0	0	0	0	0	0	0·4	1
Total		58		89		102		93		135		171		249
30-valent	91·4	53	94·4	84	94·1	96	100	93	96·3	130	93·6	160	93·6	233

Erythromycin resistance is known to be high in certain countries. In France, a total of 22·4% of isolates were erythromycin-resistant; of these, 26·4% collected from children carried *mefA*. Furthermore, about half of these had the *emm4* genotype [23]. In Spain, 17% of the invasive isolates were resistant to erythromycin, and half of them harboured the *mefA* gene, with the most frequent clone carrying the *emm4* genotype [24]. In China, from 2005 to 2008, although 97·6% of strains isolated from children were resistant to erythromycin, there was no isolate harbouring *mefA* [25], indicating that the increase in the *mefA*-positive *emm1* genotyped isolates is rare.

A similar increase in the erythromycin resistance was also observed about 40 years ago in Japan [26], when the frequency of erythromycin-resistant GAS increased from 70% to 80%. In this study, we found that the rate of erythromycin resistance in isolates was nearly 70% in 2011. An accurate comparison of these two results is not possible since the previous

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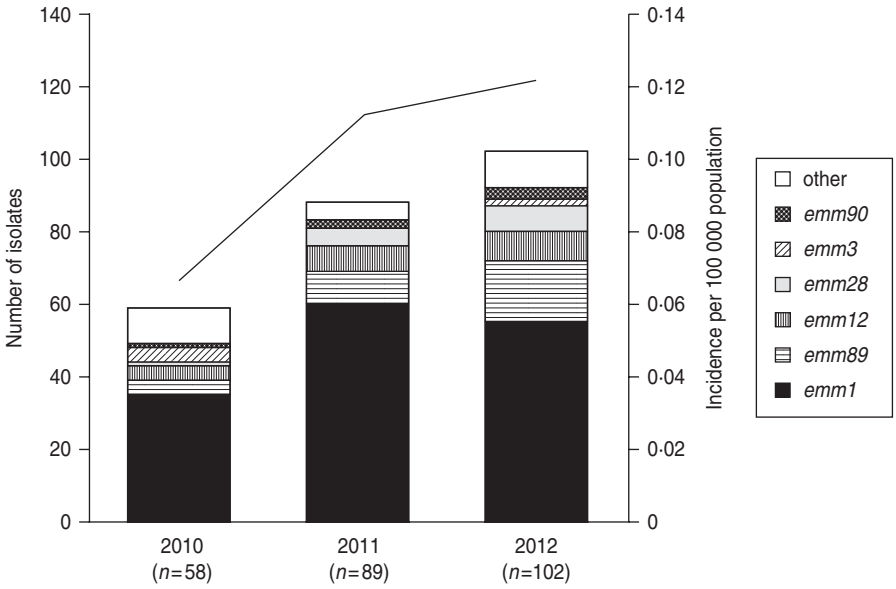

Fig. 1. The number of *emm* genotypes of strains isolated from patients of streptococcal toxic shock syndrome (STSS) in 2010, 2011, and 2012. Values in parentheses indicate the number of isolates collected annually. The line graph indicates the rate of incidence of group A streptococcus (GAS)-induced STSS per 100 000 individuals in each year (2010, 0·066; 2011, 0·113; 2012, 0·121). The number of GAS-induced STSS cases reported in each year is also recorded: 2010, $n=86$; 2011, $n=143$; 2012, $n=154$.

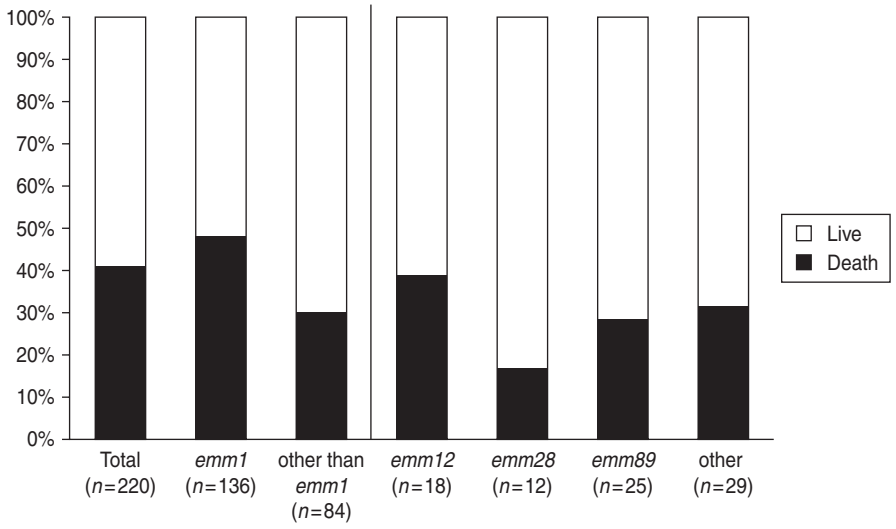

Fig. 2. Mortality rates (in %) according to the different *emm* types. Values in parentheses indicate the number of isolates that outcome has become clear for in each *emm* genotype. Y-axis indicates the mortality rate in each *emm* genotype.

studies did not use STSS isolates. In addition, the majority of erythromycin-resistant isolates found in this study were of the *emm1* genotype, whereas those reported previously were mainly of the T12 serotype. The T12 serotype is probably equivalent to *emm12*; these findings suggest that the recent increase in erythromycin resistance is distinct from that reported previously.

Clindamycin-resistant genotypes

A small number of constitutive clindamycin-resistant isolates were also found. All the erythromycin-resistant strains [isolated in 2010, three (5·2%); 2011, seven (7·9%); 2012, 11 (10·8%)] carrying the *ermB* gene were also clindamycin resistant. Similar to the previous results [11], all the isolates carrying

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Table 3 *MIC_{50} and MIC_{90} values and the antimicrobial resistance (%) for 249 clinical isolates of* Streptococcus pyogenes

Antimicrobial agent	Breakpoint for resistance* (mg/l)	Number of resistant isolates (%)	Range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)
Penicillin G	⩾0·25	0 (0)	0·008–0·015	0·008	0·008
Ampicillin	⩾0·5	0 (0)	0·015–0·06	0·03	0·03
Cefotaxime	⩾1	0 (0)	⩽0·008–0·03	0·015	0·015
Erythromycin	⩾1	160 (64·3)	⩽0·06 to ⩾16	⩾16	⩾16
Clindamycin	⩾1	21 (8·5)	0·06 to ⩾8	0·12	0·25
Linezolid	⩾4	0 (0)	1–2	2	2

MIC, Minimum inhibitory concentration.
* Breakpoints for antimicrobial resistance were determined according to the guidelines set by the Clinical and Laboratory Standards Institute (M100-S23).

Table 4. *The number of isolates with genes for erythromycin resistance collected annually from 2010 to 2012, and the types of emm genotype detected*

	2010			2011			2012			
	*N**	Genotype	*n*†	*N**	Genotype	*n*†	*N**	Genotype	*n*†	Total
mefA	31			53			54			138
		emm1	29		*emm1*	50		*emm1*	53	
		emm4	2		*emm4*	1		*emm12*	1	
					emm12	2				
ermA	1			0			0			1
		emm58	1							
ermB	3			7			11			21
		emm1	1		*emm1*	1		*emm12*	5	
		emm12	1		*emm12*	2		*emm28*	5	
		emm28	1		*emm28*	4		*emm75*	1	
Total resistant	35 (60·3%)			60 (67·4%)			65 (63·7%)			160 (64·3%)
Sensitive	23			29			37			89
Total	58			89			102			249

* Values indicate the number of isolates with resistance genes collected in each year.
† Values indicate the number of isolates with resistance gene collected for every *emm* genotype.

the *ermB* gene were resistant to both erythromycin and clindamycin, and carried neither the *mefA* nor the *ermA* gene. The *ermA* gene was detected in all the isolates that presented inducible macrolide/clindamycin-resistant phenotypes. The *emm* genotypes of constitutive clindamycin-resistant isolates were found to be *emm1, emm12, emm28*, and *emm75* (Table 3). Compared to the 2010 data, the number of *emm28* isolates increased in 2011 and the number of *emm12* and *emm28* isolates increased in 2012. The increase in clindamycin resistance in the number of *emm12* and *emm28* isolates may be one of the driving forces behind the increase in STSS.

In France, erythromycin-resistant strains of *Streptococcus pyogenes* increased during 2002–2003. 69·4% of them harboured the *ermB* gene and were resistant to clindamycin. This increasing drug resistance in France is known to be associated with the *emm28* genotype [23]. Moreover, the increase in the number of *emm28* isolates may be related to the increase in clindamycin resistance found in France.

Sequence variation in *emm* and *sic* genes

The number of *mefA*-positive *emm1* isolates has recently escalated. To examine whether this increase is due to clonal expansion, we first examined the *emm1* subtype of these isolates. Out of the 132 *mefA*-positive *emm1* isolates, 131 isolates were *emm1·0*, and one isolate was *emm1·18*.

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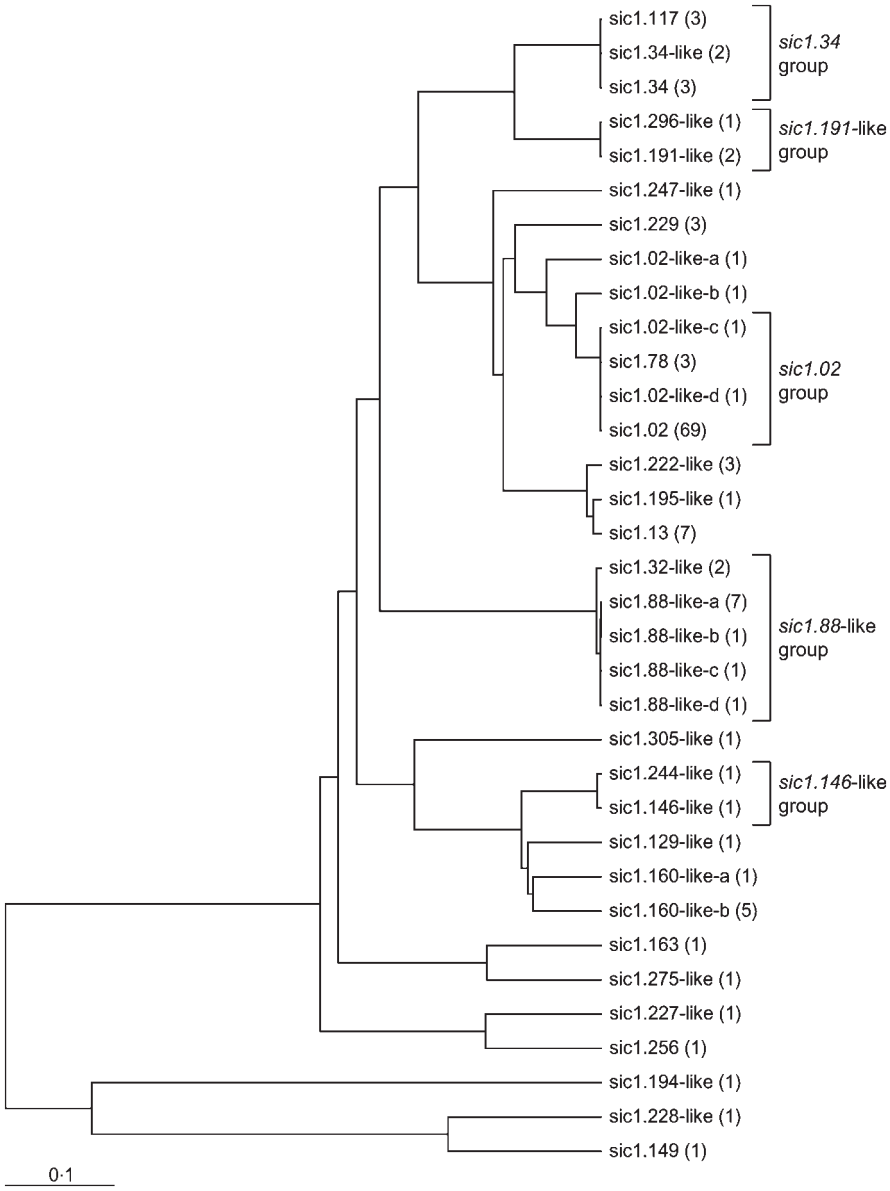

Fig. 3. Sequence comparison of the *sic* alleles from streptococcal toxic shock syndrome isolates ($n = 131$) collected in Japan. The *sic* gene was utilized to create a phylogenetic tree. The numbers in parentheses indicate the number of isolates in each *sic* genotype.

The sequence variation in the *sic* gene is useful for differentiating between the *emm1* genotyped isolates [14]. We investigated the *mefA*-positive *emm1·0*-genotyped isolates in detail by sequencing the *sic* gene. Of the 131 isolates, the *sic* genes from 91 isolates (69·5%) have already been reported (nine types), and those from 40 isolates (30·5%) were new *sic*-types (25 types). There were 34 *sic*-types in total, and among these, *sic1·02* was the most abundant allele found in this study (69 isolates) (Fig. 3). The number of *sic1·02*-typed isolates (2010, 20 strains; 2011, 26; 2012, 23) accounts for about half of the total isolates every year (2010, 28 strains; 2011, 50; 2012, 53). Phylogenetic analyses identified five groups (*sic1·02, sic1·34, sic1·88*-like, *sic1·146*-like, *sic1·191*-like groups), the most abundant group being *sic1·02*, followed by *sic1·88*-like and *sic1·34* (Fig. 3). In Finland, *sic1·01* was found to be the most prevalent in the invasive isolates [27], but the *sic1·01*-like and the *sic1·01* genotyped strains were not isolated in this study, suggesting that the *sic* genotype may be geographically different.

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CONCLUSIONS

We surveyed 249 strains isolated from patients with STSS during 2010–2012 in Japan. Isolates with the *emm1* genotype remained dominant throughout 2010–2012, as reported in the previous years. Six genotypes (*emm1*, *emm89*, *emm12*, *emm28*, *emm3*, *emm90*) constituted more than 90% of all the STSS isolates. As the number of STSS cases increased, the number of *emm1*, *emm89*, *emm12*, and *emm28* isolates also increased. Therefore, the increase in the rate of incidence of STSS can be attributed to the increase in the number of cases associated with these specific genotypes.

APPENDIX

Other members of the Working Group for Beta-haemolytic Streptococci in Japan

Y. Morimoto (Hokkaido Institute of Public Health), Y. Sakamoto (Sapporo City Institute of Public Health), H. Takenuma (Aomori Prefectural Institute of Public Health and Environment), K. Iwabuchi (Research Institute for Environmental Sciences and Public Health of Iwate Prefecture), T. Konno (Akita Prefectural Research Centre for Public Health and Environment), I. Goto (Miyagi Prefectural Institute of Public Health and Environment), J. Seto (Yamagata Prefectural Institute of Public Health), M. Hosoya (Niigata Prefectural Institute of Public Health and Environmental Sciences), G. Kobayashi (Niigata City Institute of Public Health and the Environment), T. Takagi (Ibaraki Prefectural Institute of Public Health), H. Naitou (Tochigi Prefectural Institute of Public Health and Environmental Science), C. Nagashima (Utsunomiya City Institute of Public Health and Environment Science), Y. Kawai (Gunma Prefectural Institute of Public Health and Environmental Sciences), N. Shimada (Saitama Institute of Public Health), Y. Hachisu (Chiba Prefectural Institute of Public Health), Y. Matsumoto (Yokohama City Institute of Health), Y. Kojima (Kawasaki City Institute for Pubric Health), A. Sasano (Sagamihara City Laboratory of Public Health), H. Kasahara (Nagano Environmental Conservation Research Institute), K. Takai (Shizuoka Institute of Environment and Hygiene), A. Tomita (Shizuoka City Institute of Environmental Sciences and Public Health), Y. Tsuchiya (Hamamatsu Health Environment Research Centre), H. Ueno (Saitama City Institute of Health Science and Research), M. Akanuma (Nagano City Health Office Environmental Health Laboratory), M. Matsumoto (Aichi Prefectural Institute of Public Health), K. Fukushima (Shiga Prefectural Institute of Public Health), N. Asai (Kyoto Prefectural Institute of Public Health and Environment), M. Shimizu (Kyoto City Institute of Health and Environmental Sciences), H. Mori (Osaka City Public Health Office), M. Sugimoto (Sakai City Institute of Public Health), N. Tada (Environmental Hygiene Inspection Centre of Higashiosaka City), E. Saito (Hyogo Prefectural Institute of Public Health and Consumer Sciences), S. Tanaka (Kobe Institute of Health), S. Kawanishi (Himeji City Institute of Environment and Health), S. Tanabe (Nara Prefectural Institute for Hygiene and Environment), A. Kuwata (Wakayama Prefectural Research Centre of Environment and Public Health), Y. Kanazawa (Wakayama city Institute of Public Health), Y. Hanahara (Tottori Prefectural Institute of Public Health and Environmental Science, Health and Sanitation office), M. Kurosaki (Shimane Prefectural Institute of Public Health and Environmental Science), H. Nakajima (Okayama Prefectural Institute for Environmental Science and Public Health), Y. Takeda (Hiroshima Prefectural Technology Research Institute), M. Kodama (Hiroshima City Institute of Public Health), J. Uchida (Kagawa Prefectural Research Institute for Environmental Sciences and Public Health), H. Ishida (Tokushima Prefectural Public Health Pharmaceutical and Environmental Sciences Centre), J. Matsumoto (Ehime Prefectural Institute of Public Health and Environmental Science), A. Fujito (Public Health Institute of Kochi Prefecture), S. Moroishi (Saga Prefectural Institute of Public Health and Pharmaceutical Research), and J. Kudaka (Okinawa Prefectural Institute of Health and Environment).

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DECLARATION OF INTEREST

None.

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