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Prevalence of Streptococcus Invasive Locus (*sil*) and Its Relationship with Macrolide Resistance among Group A *Streptococcus* Strains[▽]

A recent study by Bidet et al. (1) reported the molecular epidemiology of the streptococcal invasive locus (*sil*) in the group A streptococcus (GAS), an organism which caused invasive infections in French children. The authors demonstrated the prevalence of *emm* type toxin genotypes among 74 invasive GAS isolates from French children. The authors PCR amplified and characterized the locus DNA of *sil* from invasive isolates, but there were no data concerning noninvasive isolates. It seems that the invasive locus was present not only in invasive isolates but possibly also in noninvasive isolates. Therefore, we conducted a study in which our aims were (i) to examine the prevalence of *Streptococcus pyogenes* exotoxins in relationship to the *sil* gene in invasive and noninvasive isolates of GAS, (ii) to define whether *sil* was predominantly present only in invasive isolates or also in noninvasive isolates of GAS, and (iii) to characterize the relationship between GAS and macrolide resistance.

To set up our hypothesis, we examined 242 noninvasive isolates (tonsillitis, 170 isolates; rhinosinusitis, 51 isolates; and acute otitis media, 21 isolates) and 13 invasive isolates (septicemia, 5 isolates; purulent arthritis, 4 isolates; meningitis, 2 isolates; necrotizing fasciitis, 1 isolate; and peritoneal abscess, 1 isolate) of GAS, which were isolated from individual patients. *emm* typing of GAS strains was performed by DNA sequencing according to the recommendations of the Division of Bacterial and Mycotic Diseases, the Centers for Disease Control and Prevention, and the *emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm). Multiplex PCR was used for toxin gene (*speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *ssa*, and *smeZ*) profiling, as described by Schmitz et al. (5). PCR detection of the *sil* locus was performed according to the method described by Bidet et al. (1). Macrolide resistance genes of GAS were determined by the PCR methods described by Weber et al. (6). To study the degree of macrolide resistance, MICs of azithromycin to all strains were determined by broth microdilution, using the standard method (2). All the experiments were conducted in duplicate.

Among the 242 noninvasive isolates, 11.98% (29/242) harbored the *sil* gene in their genomic DNA. The *emm* types and the toxin gene profiles of *sil*-positive isolates are shown in Table 1. In noninvasive strains, the *sil* locus was detected in 9 out of 33 *emm* types found in the collection (27.27%), and 41.4% (12/29) of the *sil*-positive isolates belonged to *emm* type 4. *emm* type 4 (12 isolates), *emm* type 48 (3 isolates), and *emm* type 94 (6 isolates) represented 72.41% (21/29) of the *sil*-positive isolates. All of the *sil*-positive noninvasive isolates carried *speB* alleles, but 68.96% of strains carried *speC*. There were no significant differences between the toxin gene profile of the *sil*-positive isolates and that of the *sil*-negative isolates, except for *smeZ*, which was 10.3% of the *sil*-negative isolates but 31% of the *sil*-positive noninvasive isolates. Seventy-five percent of *emm* type 4, 75% of *emm* type 48, 100% of *emm* type 94, 100% of *emm* type 53, 100% of *emm* type 54, and 100% of *emm* type 102 isolates harbored the *sil* gene in their DNA.

Although we used limited numbers of invasive isolates, 15.4% of the invasive GAS isolates harbored the *sil* gene, which is consistent with data from a previous study of invasive strains, which showed that 16% carried the *sil* gene (1). One hundred percent of *emm* type 87 and 100% of *emm* sequence type 1732 were positive for the invasive locus. Thirty percent of the *sil*-negative invasive isolates carried *speA* alleles, but all *sil*-positive isolates were negative for the *speA* gene. All strains were positive for the *speB* gene. Fifty percent of the *sil*-positive isolates were positive for *speC*, but 30% of the *sil*-negative isolates were positive for *speC*. There is no statistical significance in the prevalence of the *sil* gene among invasive and noninvasive isolates (Fisher's exact test, $P = 0.499$).

Among 255 invasive and noninvasive isolates, 16.86% (3 were invasive, and 40 were noninvasive; total, 43/255) of the isolates were azithromycin resistant and were positive for macrolide-resistant genes (Table 2). Among these strains, 65.12% (28/43), 13.95% (6/43), and 20.93% (9/43) of the strains possessed the *mef*(A), *erm*(B), and *erm*(TR) genes, respectively. All *sil*-positive isolates were sensitive to azithromycin and were negative for macrolide resistance genes (Fisher's exact test, $P < 0.006$).

From these result, we concluded that *sil* is present not only among invasive isolates but also among noninvasive isolates, with similar prevalences (15.4% versus 11.98%, respectively). To our knowledge, this is the first report to show the prevalence rates of *sil* in both invasive and noninvasive isolates of GAS in Japan. The predominant *emm* types that harbored *sil* were *emm* type 4, *emm* type 94, and *emm* type 48. Hidalgo-Grass et al. identified *sil* in the invasive serotype M14 clone, the organism that caused necrotizing fasciitis in Israel (3). In our study, *sil* was absent from *emm* type 3 isolates, a finding comparable to that in a previous study and associated with GAS invasive diseases worldwide (3). The *sil* locus was confirmed by direct sequencing of several representative PCR-

TABLE 1. Characteristics of streptococcal toxin gene profile of invasive and noninvasive *sil*-positive isolates[a]

Isolate type	*emm* type	Sequence type	No. of isolates	Pyogenic exotoxin				
				speA	*speB*	*speC*	*speH*	*smeZ*
Noninvasive	1		1	+	+	−	−	+
	1		1	−	+	−	−	+
	4		4	+	+	+	−	−
	4		2	−	+	+	−	−
	4		5	−	+	+	−	+
	4		1	−	+	+	+	+
	11		1	−	+	+	−	−
	48		3	−	+	+	−	−
	53		1	−	+	+	+	+
	54		1	−	+	+	−	−
	75		2	−	+	−	−	−
	94		4	−	+	−	−	−
	94		1	−	+	+	−	−
	94.1		1	−	+	−	−	−
	102.2		1	−	+	+	−	−
Invasive	87		1	−	+	+	−	−
		1732	1	−	+	−	−	−

[a] Characteristics of streptococcal toxin gene profile indicating the presence (+) and absence (−) of invasive and noninvasive *sil*-positive isolates.

TABLE 2. Relationship between *sil*-positive and macrolide-resistant genes and invasive and noninvasive GAS[a]

sil gene	No. of isolates				
	Macrolide resistance gene				Total
	Positive			Negative	
	mef(A)	*erm*(B)	*erm*(TR)		
Positive	0	0	0	31	31
Negative	28	6	9	181	224
Total	28	6	9	212	255

[a] Significant differences are based on a Fisher's exact test P value of <0.006.

amplified products and comparing those with the previous sequence. The overall prevalence of the *sil* locus in invasive isolates was the same as that from a previous study (16% versus 15.4%, respectively) (1). Up to now, there was no study which showed the status of noninvasive strains with the *sil* gene. When we examined noninvasive strains, the *sil* gene was found in 12% of isolates, which is not a remarkably different rate from that found in invasive isolates. All *sil*-positive isolates were negative for macrolide resistance genes, which were irreversibly important for clinical practice. Future studies should focus on a better understanding of the role of *sil* in the pathogenesis of GAS infection and its relationship with macrolide resistance. A recent candidate vaccine based on the M protein failed to elicit antibodies to serotype M4, and *sil*-encoded proteins might represent alternative vaccine targets for this serotype (4). The results of this study should contribute to a better understanding of the pathogenesis of GAS, as well as the epidemiology of GAS-associated disease, and to the establishment of methods for the prevention of diseases caused by GAS in Japan.

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