Prevalence of Streptococcus Invasive Locus (sil) and its Relationship with Macrolide Resistance among Group A Streptococcus

A recent study reported the molecular epidemiology of the Streptococcal invasive Locus (sil) in Group A streptococci (GAS) causing invasive infections in French Children by Bidet et al (1). The authors demonstrated about the prevalence of emm-toxin genotypes among 74 invasive group A streptococcal (GAS) isolates in French children. The authors PCR amplified and characterized the DNA locus of sil of invasive isolates but there were no data concerning noninvasive isolates. It seems that invasive locus was not only present in invasive isolates but also might be in noninvasive isolates. Therefore we conducted a research study, and the aims of the study was (1) to examine the prevalence of Streptococcus pyogenes exotoxins regarding sil gene in invasive and noninvasive isolates of group A Streptococcus; (2) to define whether sil was predominantly present only in invasive isolates or also in noninvasive isolates of GAS; and (3) to character its relationship with macrolide resistance.
To set up our hypothesis we examined 242 (tonsillitis; 170 strains, rhinosinusitis; 51 strains and AOM; 21 strains) noninvasive and 13 (septicemia; 5 strains, arthritis purulent; 4 strains, meningitis; 2 strains, necrotizing fascititis; 1 strain, peritoneal abscess; 1 strain) invasive strains of GAS which were isolated from individual patients. *emm* typing of GAS strains was performed by DNA sequencing according to the recommendation of the Division of Bacterial and Mycotic Diseases, Center for Diseases Control and Prevention, and using the *emm* sequence database (www.cdc.gov/ncidod/biotech/strep/doc.htm). Multiplex PCR was used for toxin gene profiling (*speA, speB, speC, speF, speG, speH, speJ, ssa*, and *smeZ*) as described by Schmitz et al. (5). PCR detection of the *sil* locus was performed according to Bidet et al (1). Macrolide resistance genes of GAS were determined by PCR methods described by Weber et al (6). To study the degree of macrolide resistance, MICs of all strains to azithromycin were determined by broth microdilution using the standard method (2). All the experiments were conducted in duplicate.

Among 242 noninvasive isolates 11.98% (29/242) harbored *sil*-gene in their genomic DNA. The *emm* type and the toxin gene profile of
sil-positive isolates were 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 sil-positive isolates belonged to emm type 4. emm 4 (12 isolates), emm 48 (3 isolates) and emm 94 (6 isolates) represented 72.41% (21/29) of sil-positive isolates. All sil-positive noninvasive isolates carried speB alleles but 68.96% strains carried speC. There was no significance difference of toxin gene profile between sil-positive and negative isolates except smeZ, which was 10.3% in sil-negative isolates but 31% in sil-positive in noninvasive isolates. Seventy five percent (emm 4), 75% (emm 48), 100% (emm 94), 100% (emm 53), 100% (emm 54) and 100% (emm 102) harbored sil gene in their DNA. Although we used limited numbers of invasive isolates, 15.4% of invasive GAS isolates harbored sil gene that is consistent with the previous study in invasive strains which showed 16% sil gene (1). Hundred percent (emm type 87) and 100% (emm st 1732) were positive for invasive locus. Thirty percent sil-negative invasive isolates carried speA alleles but all sil-positive isolates were negative for speA gene. All strains were positive for speB gene. Fifty percent sil-positive isolates were positive for speC but
30% sil-negative isolates were positive for speC. There is no statistical significance in the prevalence of 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, noninvasive; total; 43/255) 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) strains were possessed mefA, ermB and ermTR 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 not only present among invasive isolates but also in noninvasive isolates, with similar prevalence (15.4% versus 11.98%). In our knowledge this is first report to show the prevalence of sil in both invasive and noninvasive isolates in GAS in Japan. The predominant emm types that harbored sil were emm 4, emm 94 and emm 48. Hidalgo-Grass et al. identified sil in the invasive serotype M14 clone causing necrotizing fasciitis in Israel (3). In our study, sil was absent from emm type 3 and in comparable to previous study, which are associated
with GAS invasive diseases worldwide (3). The *sil* locus was confirmed by
direct sequencing of several representatives PCR amplified products
compared with the previous sequence. The overall prevalence of *sil* locus
in invasive (16% vs 15.4%) isolates was same compared with previous
study. Up to now there is no study which shows the status of noninvasive
strains concerning *sil* gene. When we examined noninvasive strains, *sil*
gene was found 12% of isolates which is not remarkably different from the
rate in invasive isolates. All *sil*-positive isolates were negative for
macrolide resistance genes which were irreversibly important for clinical
practice. Future study should focus on better understanding the role of *sil* in
pathogenesis of GAS and its relationship with macrolide resistance. A
recent candidate vaccine based on 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 prevention of diseases caused by GAS in Japan.
References


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Table 1: characteristics streptococcal toxin gene profile of *Sil*-positiv isolates invasive and noninvasive. +: present  -: absent.
Table 2: Relationship between *sil*-positive and macrolide resistant gene to invasive and noninvasive GAS.  \( P<0.006 \) (Fisher’s exact test)

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<th></th>
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