Short Communication


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SUMMARY: Group G Streptococcus strains isolated from patients with severe invasive infections in the period 2002–2008 were surveyed and their prevalence compared with that observed in the period 1995–2001 in Japan. Strains with genotypes stg485, stg6792, stc36, stg6, and stg652 were isolated in both periods, whereas various new genotypes appeared in 2002–2008 and some genotypes found in 1995–2001 were not found subsequently, thus indicating a change in the prevalent genotyped strains causing severe invasive streptococcal infections.

Group G streptococci (GGS), which are common components of the normal flora of human skin, pharynx, and gastrointestinal tract (1), can cause pharyngitis, skin and soft tissue infection, septic arthritis, bacteremia, and endocarditis. Since the late 1980s, streptococcal toxic shock syndrome (STSS) caused by Streptococcus pyogenes (group A streptococci [GAS]) has become a serious problem in both developed and developing countries. The characteristic symptoms of STSS progress very rapidly and are fulminating from the onset. Indeed, patients can develop necrotizing fasciitis, acute kidney failure, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), and multiorgan failure, leading to shock and death, within the space of a few hours. GGS identified as Streptococcus dysgalactiae subsp. equisimilis is also known to cause STSS (2,3), with ourselves and others having reported dozens of such cases to date in Japan (4,5). The first case of STSS caused by S. dysgalactiae subsp. equisimilis was occurred in 1995 and we described the characteristics of severe invasive GGS isolates in the period 1995–2001 (4). In that study we proposed that GGS carrying a particular emm genotype does not necessarily cause invasive disease and that clonal expansion of GGS is unable to account for the emergence of disease. The emm1-genotype strains were dominant among the severe invasive GAS isolated in Japan, although there was a slight reduction in the ratio of emm1 to other genotypes after 2001 (6). The emm genotype may change in GGS isolates from patients with severe invasive infections. In this study, we compare the characteristics of severe invasive GGS isolated in Japan in the periods 2002–2008 and 1995–2001.

The activity of the Working Group for β-hemolytic streptococci in Japan is based on a network between the National Institute of Infectious Diseases and prefectural public health institutes (4), with seven branches of the reference center being located at the public health institutes of Fukushima, Toyama, Tokyo, Kanagawa, Osaka, Yamaguchi, and Oita. Clinical isolates and the available data on streptococcal infections are transferred from cooperative hospitals throughout Japan to the public health institutes and then on to the National Institute of Infectious Diseases. The diagnostic criteria for severe invasive GGS infections are based on the principle for necrotizing fasciitis and STSS (7) (characterized by two of the symptoms listed in Table 1) and on analysis of the GGS isolated from sterile body sites.

Twenty-seven cases of severe invasive infection due to GGS were reported in 2002–2008 (Table 1). Less than half (44.4%) of patients were male. The patients’ ages ranged between 29 and 91 years, and the average age (66.1 years) was higher than that of the STSS patients in 1995–2001 (56.0 years) (8; unpublished data). The average age of female patients (73.1 years) was sig-

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significantly higher than that of male patients (57.3 years), and the mortality rate was 45.8% (54.6% male, 38.5% female). Of the 24 cases with a detailed description of the disease history, 75.0% (18/24) had at least one or more underlying disease, including cancer (4/24, 16.7%), diabetes mellitus (3/24, 12.5%), cardiac disease (3/24, 12.5%), rheumatoid arthritis (2/24, 8.3%), cirrhosis (2/24, 8.3%), hypertension (2/24, 8.3%), renal disease (2/24, 8.3%), and one patient for each of cerebrovascular disease, Down syndrome, epilepsy, schizophrenia, probable syringomyelia, myelitis, lymphatic edema, systemic lupus erythematosus, gout, and osteoarthritis. Twenty-two patients had clinical shock, and varying numbers had renal impairment, DIC, liver involvement, ARDS, a generalized erythematous maculopapular rash, or soft-tissue necrosis (Table 1). A total of 28 GGS strains, which consisted of 26 isolates from 26 cases and 2 distinct genotyped isolates from the same case (NIH359), were examined (Table 1). All GGS isolates were identified as S. dysgalactiae subsp. equisimilis using the Streptex-Kit (Mitsubishi Chemical Medience, Tokyo, Japan) and API 20 Strep kits (BioMerieux Vitek, Hazelwood, Mo., USA). GGS were isolated from blood samples in 19 (76%) of the 26 cases, from soft tissues in 6 cases (16%), and from the abscess specimen in 1 case (8%). The site of bacterial isolation was unknown in one case.

GGS express the cell surface M-like protein and at least 23 forms of the emm-like gene (stg); a molecular approach for identifying the stg genes has been documented (9) (http://www.cdc.gov/ncidod/biotech/strep/streptindex.htm). We determined the dominant emm genotypes among our 28 severe invasive GGS isolates by sequencing the emm genes (Table 1), as described previously (4). Twelve kinds of emm genotyped strains, namely stg485 (4/28), stg6792 (4/28), stc36 (4/28), stg2078 (3/28), stg652 (2/28), stg4974 (1/28), stc74a (1/28), and stc1400 (1/28), were isolated and compared with the emm genotypes identified in 1995–2001 (4). Strains with the stg485, stg6792, stc36, stg6, and stg652 genotypes were isolated in both periods, although the number of stg6792 genotype strains was higher in 2002–2008 than in 1995–2002. The genotyped strains stg10, stg4974, stc1400, and stc1400 have emerged since 2002, whereas strains with the stg11, stg880, and stg840 genotypes, which were isolated before 2001 (4), have not been detected since 2002. These findings suggest that several emm-genotype strains cause severe invasive infections and that the genotypes of isolated strains are changing.

The presence of specific virulence genes, scpA, ska, slo, sagA, sla, speA, speB, speC, speG, speH, speI,
speL, speL (M3), speL (M18), and speM, was examined by PCR with specific primers, as described previously (4). As was the case in our previous report (4), all isolates carried the scpA, ska, slo, and sagA genes (Table 1). Likewise, all strains were negative for sla, speA, speB, speC, speH, speI, speJ, speL (M3), speL (M18), and speM (data not shown). Twelve (42.9%) of the GGS isolates carried the speG gene (Table 1). In comparison with the strains isolated before 2002, no difference was found in terms of the kinds of virulence genes carried.

We did not perform an antimicrobial susceptibility test in our previous study (4), therefore in this study we analyzed the antimicrobial susceptibility of the isolates from both periods to 11 drugs, namely penicillin G (PCG), ampicillin (ABPC), cefazolin (CEZ), cefotaxime (CTX), imipenem (IPM), panipenem (PAPM), erythromycin (EM), clindamycin (CLDM), telithromycin (TEL), linezolid (LZD), and ciprofloxacin (CPFX) by the broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (10). The resistance breakpoint of each drug, except CEZ, IPM, PAPM, TEL, and CPFX, was in line with the recommendations of the CLSI (10). The breakpoint for CEZ was equivalent to the CLSI-recommended breakpoint for CTX. Similarly, the breakpoints for IPM and PAPM were equivalent to, and in line with, the CLSI recommendations for Streptococcus pneumoniae, as was the breakpoint for TEL. The breakpoint for CPFX was equivalent to that of the CLSI-recommended breakpoint for levofloxacin. The resistance genes (ermA, ermB, and mefA) of EM-resistant strains were screened by PCR using previously published primer sequences (11,12). All isolates were susceptible to PCG, ABPC, CEZ, CTX, IPM, PAPM, TEL, LZD, and CPFX. Three strains (NIH265, NIH329, and NIH482; 11.1%) isolated in the period 2002–2008 were resistant to EM, and two (NIH329, NIH482; 7.41%) of these were also resistant to CLDM (Table 2). The breakpoint for tetracycline. Only three isolates (NIH126, NIH181, and NIH482) among the six EM-resistant strains, all of which harbored the tetM gene, were resistant to MINO (Table 2). These results suggest that NIH482 may have a similar system to be susceptible to TEL.

We finally found a change in the prevalent genotypes of EM-resistant isolates causing severe invasive GAS infections in Japan, particularly in 2006 and 2008. Strains with the stg485, stg652, stg840, and stg652 genotypes were isolated in both 1995–2001 and 2002–2008, although no dominant emm genotype responsible for severe GGS-related invasive infections has been identified in Japan. Finally, we found a change in the prevalent genotypes of strains causing severe invasive streptococcal infections between the two periods studied.

Appendix. The following are the other members of The Working Group for β-hemolytic Streptococci in Japan: Akihito Wada (National Institute of Infectious Diseases, Tokyo), Satoko Matsunaga (National Institute of Infectious Diseases, Tokyo) Tsuneaki Kawai (Sapporo City Institute of Public Health, Hokkaido), Atsushi Waguri (Aomori Prefectural Institute of Public Health and Environment, Aomori), Takayuki Konno (Akita Prefectural Research Center for

Table 2. Characteristics of erythromycin-resistant strains

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Isolation year</th>
<th>Antimicrobial susceptibility</th>
<th>Resistance gene</th>
<th>emm allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EM</td>
<td>CLDM</td>
<td>TEL</td>
</tr>
<tr>
<td>183</td>
<td>1998</td>
<td>≥4</td>
<td>≥4</td>
<td>16</td>
</tr>
<tr>
<td>126</td>
<td>2000</td>
<td>≥4</td>
<td>0.12</td>
<td>0.5</td>
</tr>
<tr>
<td>181</td>
<td>2001</td>
<td>≥4</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>265</td>
<td>2004</td>
<td>≥4</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>329</td>
<td>2006</td>
<td>≥4</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>482</td>
<td>2008</td>
<td>≥4</td>
<td>≥4</td>
<td>0.25</td>
</tr>
</tbody>
</table>

1): µg/ml
2): +, positive; –, negative
EM, erythromycin; CLDM, clindamycin; TEL, telithromycin; MINO, minocycline.
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Conflict of interest None to declare.

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