Short Communication

Comparative Analysis of Penicillin-Susceptible and Non-Susceptible Isolates of Group B Streptococci by Multilocus Sequence Typing

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SUMMARY: Since Group B *Streptococcus* (GBS, *Streptococcus agalactiae*) clinical isolates are believed to be uniformly susceptible to β -lactams, penicillin G has been used as the first-line agent for the prevention and treatment of GBS infections. However, the existence and characteristics of GBS isolates with reduced penicillin susceptibility (PRGBS) have recently been reported in Japan. Moreover, the sequence type (ST) 458 is predominant among the PRGBS in Japan. Although the majority of the PRGBS isolates in Japan have been recovered from respiratory specimens of adults, no information on the genotype of these isolates is available. Therefore, whether ST458 predominates among GBS isolates obtained from such specimens is not known. In this study, we characterized the STs of 38 penicillin-susceptible GBS isolates (PSGBS) recovered from respiratory specimens and compared them to the reported PRGBS STs. ST458, the predominant ST among the PRGBS isolates studied (10/19, 53%), was not found in the PSGBS isolates. Thirty-six PSGBS isolates belonged to the ST1/19/10 group (includes 6 different STs), and the remaining 2 isolates belonged to that of ST23. Further, the PRGBS isolates were divided into the ST1 (3 STs), and ST23 (2 STs) groups. ST458 was not predominant among the PSGBS isolates recovered from respiratory specimens in Japan and may therefore be specific to the PRGBS. Thus, the ST distribution of the PRGBS isolates does not reflect that of the PSGBS.

Group B *Streptococcus* (GBS, *Streptococcus agalactiae* [S. *agalactiae*]) is often isolated from the digestive or lower genital tract and is an important pathogen. GBS is the main cause of sepsis and meningitis in neonates. It also causes serious infections in pregnant women, the elderly, and people with underlying diseases (1,2). GBS clinical isolates are believed to be uniformly susceptible to β -lactams, and penicillin G (PCG) provides the first-line of prevention and treatment against GBS infections. However, Kimura et al. recently reported the existence and characteristics of GBS isolates with reduced penicillin susceptibility (PRGBS) in Japan (3). PRGBS isolates were later reported in the US and Canada as well (4–6). We had previously reported that the PRGBS tends to be resistant to other drugs, that is, fluoroquinolones and macrolides (7). We also showed that a clinical isolate of PRGBS turned highly cephalosporin-resistant through the acquisition of amino acid substitutions in the penicillin-binding proteins PBP1A and PBP2X (8). Therefore, the PRGBS is a potentially significant public health concern.

Multilocus sequence typing (MLST) analyses of PRGBS isolates in Japan and the US have been reported. These studies reported that the 28 strains of PRGBS

found in Japan were divided into 7 sequence types. Eleven (39%) belonged to the ST458, which was a novel finding in that study (9). Moreover, the ST1 group (includes ST1, and STs similar to ST1), made up of 5 different STs including the ST458, was found to be predominant (23/28, 82%) (9). Meanwhile, the 4 PRGBS isolates recovered in the US belonged to the same ST (ST19) (4). Although PRGBS isolates recovered from a sacral decubitus ulcer have also been reported (10), most of the PRGBS isolates in Japan were recovered from adult respiratory specimens (9). However, MLST data for penicillin-susceptible GBS (PSGBS) isolates from adult respiratory specimens are limited (11). Specifically, no information is available on GBS isolates from adult respiratory specimens in Japan. Therefore, we determined the STs of 38 PSGBS isolates recovered from independent adult sputum samples in Japan, and compared them to the reported PRGBS STs, to elucidate the development of penicillin resistance in the GBS.

Toward this, we selected 19 PRGBS (from isolates patients 20–64 years [$n = 4$], and ≥ 65 years [$n = 15$] old) and 38 PSGBS (from patients 20–64 years [$n = 12$], and ≥ 65 years [$n = 24$] old) isolates that were acquired from various Japanese medical institutions during the period 2001–2008. The isolates were recovered chiefly from respiratory specimens.

MICs of penicillin G were determined by the agar dilution method using *S. pneumoniae* (ATCC 49619) as the quality control, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (12).

The STs of the 38 PSGBS isolates were determined as described previously (9). Chromosomal DNA was ex-

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Fig. 1. Stacked bar graphs of PRGBS and PSGBS STs. Graphs A and B show the STs of PRGBS and PSGBS, respectively.

tracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, US) with mutanolysin, according to the manufacturer's instructions. The MLST was performed as described previously (13). Seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkl*) were PCR-amplified with the high fidelity PrimeSTAR HS DNA polymerase (Takara, Otsu, Japan). This was followed by amplicon purification using the Wizard SV Gel and PCR Clean-Up System (Promega) per manufacturer's instructions. Next, the nucleotide sequences were determined using the BigDye Terminator V3.1 on the Applied Biosystems (Waltham, MA, US) 3130xl and 3730xl instruments. One allele type was assigned to each sequence according to the MLST database for *S. agalactiae* (<http://pubmlst.org/sagalactiae/>). STs were then identified by combining the 7 allele types, and linkages were analyzed by eBURST V3 (<http://eburst.mlst.net/>).

We selected 19 of 28 previously analyzed PRGBS isolates (9,14), which were recovered during the same period as that of the 38 PSGBS isolates in this study. The ST458 was predominant (10/19, 53%) in the PRGBS isolates, followed by ST1 (5/19, 26%) (Figure 1A). All the 38 PSGBS isolates were susceptible to PCG (MIC range, 0.03–0.06 µg/mL). The PSGBS STs are shown in Figure 1B. Interestingly, the ST458 was absent in the PSGBS isolates tested in this study, while ST1 was the most common (25/38, 66%). The frequencies of ST19, ST10, and ST23 were 4 (11%), 4 (11%), and 2 (5%), respectively. Furthermore, ST12, ST153, and

ST573 were identified only once. Notably, the ST573 is a novel ST identified in this study.

According to the eBURST analysis, 6 PSGBS STs (ST1, ST153, ST19, ST10, ST12, and ST573) are genetically related, as illustrated in Figure 2. Therefore, these STs formed the ST1/19/10 group. The ST153 differed from ST1 by a single allele, and so did the ST12 and ST573 from ST10. The PRGBS STs were also divided into 2 groups: ST1 (ST458, ST1, and ST358) and ST23 (ST23 and ST464).

Figure 1 shows the STs of the 38 PSGBS and 19 PRGBS isolates. The PRGBS isolates in Japan can be classified into at least 2 groups: ST1 (16/19, 84%) and ST23 (3/19, 16%). In the present study, we divided the PSGBS STs into the ST1/19/10 (36/38, 95%) and ST23 (2/38, 5%) groups.

This investigation demonstrates that the ST distribution of the PRGBS isolates does not reflect that of the STs among the PSGBS isolates from respiratory specimens of adults in Japan. This also implies that the ST458 might be a lineage specific to the PRGBS.

However, sufficient data is not available to support this specificity conclusively.

In summary, we eliminated the possibility that the ST458 is predominant among the PSGBS isolates from the respiratory specimens obtained from adults in Japan and that the ST distribution of the PRGBS reflects that of the PSGBS isolates. Further, the PRGBS tends to be multidrug-resistant (7), and a clinical isolate of GBS became highly resistant to cephalosporin through amino

Fig. 2. eBURST analysis of STs of GBS. Numbers stand for STs, and neighbouring STs connected by a line differ at 1 allele. STs marked with an asterisk were identified previously in PRGBS in Japan. STs surrounded by squares were found in PSGBS in this study. A shows ST1/19/10 group and B shows ST23 group. STs in the figure were picked randomly from all STs.

acid substitutions in 2 PBPs (8). The nosocomial spread of a multidrug-resistant PRGBS, belonging to the ST458, has been reported (15). At present, all the STs of PRGBS belong to the ST1/19/10 or ST23 group. There is no report that the PRGBS clinical isolates belong to the ST17 group, which are often isolated from neonatal meningitides. Attention should be focused on monitoring and studying PRGBS isolates since these organisms might become a future public health concern.

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Conflict of interest None to declare.

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