Comparative analysis of penicillin-susceptible and non-susceptible isolates in group B streptococci by multilocus sequence typing


Received: September 8, 2014. Accepted: November 4, 2014

Published online: February 13, 2015
DOI: 10.7883/yoken.JJID.2014.387

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Short Communication

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


1Department of Bacteriology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan
2Department of Bacteriology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan
3Medical Microbiology Laboratory, Funabashi Municipal Medical Center, 1-21-1 Kanasugi, Funabashi, Chiba 273-8588, Japan
*Corresponding author: Mailing address: Department of Bacteriology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan. Tel: +81-52-744-2106, FAX: +81-52-744-2107, Email: koujikim@med.nagoya-u.ac.jp
Keywords

Group B Streptococcus, Streptococcus agalactiae, multilocus sequence typing, Group B Streptococcus with reduced penicillin susceptibility, Penicillin-susceptible GBS

Running title: MLST of PRGBS and PSGBS

SUMMARY: Since Group B Streptococcus (GBS, Streptococcus agalactiae) clinical isolates were believed to be uniformly susceptible to β-lactams, penicillin G has been the first-line agent for the prevention and treatment of GBS infections. However, Kimura et al. recently reported the existence and characteristics of GBS isolates with reduced penicillin susceptibility (PRGBS) in Japan. Sequence type (ST) 458 is predominant among PRGBS in Japan. However, although most PRGBS isolates in Japan have been recovered from respiratory specimens from adults, no information about genotype is available concerning GBS isolates from such specimens. Therefore, whether ST458 predominates among GBS isolates obtained from such specimens is not known. We characterised the STs of 38 GBS isolates with penicillin susceptibility (PSGBS) recovered from respiratory specimens and compared them to PRGBS STs.

ST458, the predominant ST of PRGBS (10/19, 53%), was not found in PSGBS. Thirty-six PSGBS isolates belonged to the ST1/19/10 group (6 different STs); the remaining 2 isolates belonged to ST23. PRGBS were divided between the ST1 (3 STs) and ST23 groups (2 STs).

ST458 was not predominant among PSGBS recovered from respiratory specimens in Japan and may be specific to PRGBS. The ST distribution of PRGBS does not merely reflect that of PSGBS.
Main Text

Group B Streptococcus (GBS, Streptococcus 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 is also a cause of serious infections in pregnant women, the elderly, and people with underlying disease (1, 2). Since GBS clinical isolates were believed to be uniformly susceptible to β-lactams, penicillin G has been the first-line agent for the prevention and treatment of GBS infections. However, Kimura et al. recently reported the existence and characteristics of GBS isolates with reduced penicillin susceptibility (PRGBS) in Japan (3). PRGBS was later reported in the US and Canada (4-6). We had previously reported that PRGBS tends to be resistant to other drugs, fluoroquinolones and macrolides (7), and that a clinical isolate of PRGBS became highly cephalosporin resistant through the acquisition of amino acid substitutions in PBP1A and PBP2X (8). Therefore, PRGBS may become a significant public health concern.

Multilocus sequence typing (MLST) analyses of PRGBS isolates in Japan and the US have been reported. According to these reports, the 28 strains of PRGBS found in Japan are divided into 7 sequence types. Eleven (39%) belonged to ST458, which was newly identified in that study (9). The ST1 group (“ST1 group” includes ST1 and STs similar to ST1), made up of 5 different STs including ST458, was predominant (23/28, 82%) (9). Four PRGBS isolates recovered in the US belonged to the same ST (ST19) (4). Although PRGBS isolates recovered from a sacral decubitus ulcer have been reported elsewhere (10), most PRGBS isolates in Japan were recovered from adult respiratory specimens (9). However, MLST data for GBS isolates with penicillin susceptibility (PSGBS) isolated from adult respiratory specimens are quite limited (11) and no information is available on GBS isolates from adult respiratory specimens in Japan. Therefore, we determined the STs of 38 PSGBS recovered from independent adult sputum samples in Japan and compared them to the reported STs of PRGBSs to deduce the process of PRGBS development.

We selected 19 PRGBS (patient age: 20-64 years, 4 isolates, ≥65 years, 15 isolates) and 38 PSGBS isolates (patient age: 20-64 years, 12 isolates, ≥65 years, 24 isolates) recovered during
2001–2008 from various Japanese medical institutions. The isolates were recovered mainly from respiratory specimens.

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

STs of 38 PSGBS were determined as described (9). Chromosomal DNA was extracted using the Wizard genomic DNA purification kit (Promega) with mutanolysin, and MLST was performed as described (13). Seven housekeeping genes (*adhP, pheS, atr, glnA, sdhA, glicK, and tkt*) were PCR-amplified with the high fidelity PrimeSTAR HS DNA polymerase (Takara), followed by amplicon purification using the Wizard SV gel and the PCR clean-up system (Promega). The nucleotide sequences were determined using BigDye Terminator V3.1 on the Applied Biosystems 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 identified by combining 7 allele types, and linkages were analysed by eBURST V3 (http://eburst.mlst.net/).

We selected 19 PRGBS from 28 previously analysed PRGBS (9, 14), in order to match the isolation years to the 38 PSGBS analysed in this study. ST458 was the most common one (10/19, 53%), followed by ST1 (5/19, 26%) (Fig. 1A). All the 38 PSGBS were susceptible to PCG (MIC range: 0.03 to 0.06 µg/mL). The PSGBS STs are shown in Fig. 1B. ST458, the predominant ST in PRGBS, was not found at all in PSGBS tested in the present investigation. ST1 was most common (25/38, 66%). The frequencies of ST19, ST10, and ST23 were 4 (11%), 4 (11%) and 2 (5%), respectively. ST12, ST153, and ST573 were identified only once, and ST573 was newly identified in this study.

According to the eBURST analysis, 6 PSGBS STs (ST1, ST153, ST19, ST10, ST12, and ST573) have genetic connections, as described in Figure 2. Therefore, these STs formed the ST1/19/10 group. ST153 differed from ST1 by a single allele, and ST12 and ST573 differed from ST10 by a single allele. ST23 was one of the STs belonging to the ST23 group. The PRGBS STs were also divided into 2 groups, ST1 group (ST458, ST1, and ST358) and ST23 group (ST23 and ST464).

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

This investigation would demonstrate that the ST distribution of PRGBS is not merely reflecting the population of STs among PSGBS isolated from respiratory specimens of adults in Japan. This speculation would also imply that ST458 may be a specific lineage to PRGBS.

There is not sufficient data to conclude the specificity of ST458 with respect to PRGBS. However, in this investigation, we eliminated the possibility that ST458 is predominant among the PSGBS isolated from the respiratory specimens obtained from adults in Japan and the ST distribution of PRGBS merely reflects that of PSGBS. PRGBS tends to be multidrug-resistant (7) and a clinical isolate of GBS became highly resistant to cephalosporin through amino acid substitutions in 2 PBPs (8). The nosocomial spread of multidrug-resistant PRGBSs belonging to ST458 has been reported (15). At the present, All STs of PRGBS belong to ST1/19/10 group or ST23 group and there is no report that PRGBS clinical isolates belong to ST17 group, which are often isolated from neonatal meningitides. Because PRGBS may become future public health concerns, a greater deal of and more advanced attention should be focused on monitoring and researching PRGBS.

**Acknowledgements**  This study was supported by the Ministry of Health, Labour and Welfare, Japan (grant number #H24-Shinkou-Ippan-010) and in part by a Research Grant for Medical Science from the Takeda Science Foundation (2012).

We thank Dr. Akira Okamoto and all members of Professor Arakawa’s laboratory for the critical discussion and support.

**Conflict of interest**  None to declare
REFERENCES


Figure legends

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

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.
Figure 1

(A) PRGBS (19 isolates)
- ST464: 1 (5%)
- ST23: 2 (11%)
- ST358: 1 (5%)
- ST458: 10 (53%)
- ST1: 5 (26%)

(B) PSGBS (38 isolates)
- ST23: 2 (5%)
- ST573: 1 (3%)
- ST12: 1 (3%)
- ST10: 4 (11%)
- ST19: 4 (11%)
- ST153: 1 (3%)
- ST1: 25 (66%)
Figure 2