Predominance of sequence type 1 group with serotype VI among group B streptococci with reduced penicillin susceptibility identified in Japan

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Background: Although group B Streptococcus (GBS; i.e. Streptococcus agalactiae) has been considered to be uniformly susceptible to β-lactams, GBS isolates with reduced penicillin susceptibility (PRGBS) have been reported from Japan and North America. In this study, PRGBS from Japan were characterized by multilocus sequence typing (MLST) and the results compared with data on PRGBS reported from the USA.

Methods: Twenty-eight clinical isolates of PRGBS recovered in Japan (including 22 isolates previously analysed by PFGE) were analysed by MLST and eBURST (http://eburst.mlst.net/).

Results: Twenty-three isolates were found to belong to the sequence type 1 (ST1) group (11 ST458, 7 ST1, 3 ST297, 1 ST358 and 1 ST4), while the remaining 5 isolates formed the ST23 group. Among 11 ST458 and 7 ST1 isolates, 9 and 4 were serotype VI, respectively, indicating a probable correlation between the ST1 group and serotype VI for PRGBS in Japan.

Conclusions: PRGBS in Japan could be classified into at least two ST groups, ST1 and ST23, which are genetically different from the ST19 PRGBS isolated in the USA, though five allele variations were seen between ST1 and ST19, implying a slight genetic relatedness.

Keywords: β-lactams, non-susceptible, GBS, multilocus sequence typing

Introduction

Group B Streptococcus (GBS; i.e. Streptococcus agalactiae) is a major cause of neonatal sepsis and meningitis, and also an important pathogen for elderly people and those suffering from underlying medical disorders.¹–⁵ Invasive infections caused by GBS in neonates (including very low birth weight infants) are associated with high mortality and morbidity.⁶–⁸ About 5% of GBS-infected infants die, and if they survive they often suffer from severe neurological sequelae such as mental retardation and visual and/or auditory disabilities.⁶ Penicillin generally remains the first-line agent for the treatment of GBS infections, as most strains remain susceptible.⁶–⁹ However, we recently identified and molecularly characterized several clinical GBS isolates demonstrating reduced penicillin susceptibility (PRGBS) through acquisition of multiple mutations in the penicillin-binding protein 2X (PBP2X) gene.¹⁰–¹² PRGBS was also identified subsequently by several groups in the USA,¹³ Canada¹⁴ and Japan.¹⁵ Previously we reported that all but two PRGBS isolates from Japan showed different banding patterns when analysed by PFGE using the ApaI restriction endonuclease.¹⁰,¹¹ In contrast, multilocus sequence typing (MLST) showed that each of four isolates in the USA belonged to the same sequence type (ST), namely ST19.¹³ Since it is not well investigated whether or not PRGBS belong to a specific genetic lineage, we determined the ST of 28 PRGBS isolated in Japan, including 22 isolates previously analysed by PFGE using ApaI digestion.¹⁰,¹¹

Materials and methods

The PRGBS isolates were mostly from respiratory specimens of elderly people and one strain, MRY08-1422, was from blood (Table 1). Chromosomal DNA was prepared using the Wizard genomic DNA purification kit (Promega) and MLST was performed with minor modifications as described previously.¹⁶ Amplifications of partial loci of seven housekeeping genes established by Jones et al.¹⁶ and sequence analyses were performed as...
buten discs. The MICs of penicillin for the 28 isolates were consistent with the results of the disc diffusion tests using cefti-CLSI and sequence analysis of the PBP2X gene. The results were of penicillin by the agar dilution method as recommended by the All were exactly confirmed as PRGBS by determination of the MIC

### Results

The STs and characteristics of the 28 PRGBSs are listed in Table 1. All were exactly confirmed as PRGBS by determination of the MIC of penicillin by the agar dilution method as recommended by the CLSI and sequence analysis of the PBP2X gene. The results were consistent with the results of the disc diffusion tests using cefti-butten discs. The MICs of penicillin for the 28 isolates were in the range 0.25–1 mg/L. Among the 28 clinical isolates, 26 harboured both or either of the two PRGBS-specific amino acid substitutions, Q557E and V405A, in PBP2X. Although the two remaining clinical isolates, B7 and MRY08-1422, harboured neither the Q557E nor V405A substitution in PBP2X, these clinical isolates harboured several amino acid substitutions other than Q557E and V405A in the transpeptidase domain of

### Discussion

PRGBSs have thus far been mainly isolated from respiratory specimens of elderly people in Japan. In this study the STs of 28 PRGBSs were found predominantly to belong to the ST1 group, with a minority of isolates belonging to the ST23 group. ST1 and ST23 were reported as the major STs involved in carriage and invasive infections in neonates and non-pregnant adults, and one strain, MRY08-1422, isolated from blood was assigned to ST464, and thus belonged to the ST23 group. Both ST1 and ST23 have been frequently identified among the isolates of throat flora.
Although information concerning STs isolated from respiratory specimens of elderly people is limited, the STs of PRGBSs determined in this study might reflect the fact that most PRGBSs isolated so far have tended to be from respiratory specimens of elderly people.

The most frequent ST of PRGBS found in the present study was the novel type ST458. The eBURST analysis showed that ST458 is a single allelic variant of ST1. Interestingly, among the 11 ST458 and 7 ST1 strains, 9 and 4 were serotype VI, respectively (Table 1), suggesting a probable correlation between the ST1 group and
serotype VI in the PRGBSs tested. Limited information about the correlation between serotype VI and ST is available in GBS at present, but the ST of three serotype VI GBS strains deposited in the PubMLST database (http://pubmlst.org/sagalactiae/) are all ST1. Moreover, among six GBS strains with serotype VI, four strains were reported to be ST1 and the remaining two strains were ST14 (one allelic variant of ST1) and ST13 (five allelic variant of ST1), respectively. However, ST458 clinical isolates with serotype VI may have biological characteristics similar to those of ST1, more information about the strains of ST458 will be needed to evaluate the correlation between serotype VI and the ST1-group PRGBS, together with its clinical significance.

As we reported previously, the PFGE analysis of PRGBS using ApaI showed different band patterns among the PRGBS clinical isolates tested. Moreover, phylogenetic analyses of the PBP genes of PRGBS suggested their genetically divergent origin. However, an American study reported that four PRGBSs isolated in different states all belonged to ST19, suggesting a clonal expansion of PRGBS in the USA. In the present study, however, ST19 was not found among the 28 PRGBSs isolated in Japan, and a greater variety of STs was observed among the PRGBS strains than in those isolated in the USA. We confirmed, therefore, that PRGBS in Japan could be classified into at least two ST groups, the ST1-group PRGBS, together with its clinical significance.

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Supplementary data

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References