**Multilocus sequence typing of invasive group B Streptococcus in central area of Taiwan**

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**KEYWORDS**
Invasive group B Streptococcus; Multilocus sequence typing; Taiwan

**Background:** Group B Streptococcus (GBS) (Streptococcus agalactiae) is an important pathogen in neonates, pregnant women, and adults with underlying disease.

**Methods:** Fifty clinical isolates were collected during the period 2001–2004 and analyzed by multilocus sequence typing and capsular serotyping.

**Results:** The six major sequence types (STs) identified by multilocus sequence typing were ST1, ST12, ST19, ST17, ST23, and ST10. Five major clonal complexes (CCs) and one single ST (ST61) from 11 different STs were found. CC1 (n = 14) was the most common one, followed by CC12 (n = 13), CC19 (n = 9), CC17 (n = 7), and CC23 (n = 6). The most common serotypes were serotype III, followed by Ib, V, Ia, and IV. The most invasive strains in adults belonged to ST1 (CC1) and serotype V, and those in neonates belonged to ST17 (CC17) and serotype III. In addition, ST19 was distributed in adults and neonates.

**Conclusions:** These results are similar to those of previous reports, but some geographic differences were found, suggesting that limited clonal lineages play important roles in GBS-associated diseases worldwide. Continued tracking of GBS in the population through clinical isolates is important for epidemiologic investigations and vaccine development.

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Introduction

Group B Streptococcus (GBS) (Streptococcus agalactiae) is a gram-positive, catalase-negative, β-hemolytic microorganism that is an important cause of illness in newborn infants, pregnant women, and nonpregnant adults with underlying medical conditions.1–3 In newborns, GBS infection can lead to early- or late-onset diseases.2 Early-onset disease, occurring usually within the first few hours to 1 week of life, results from aspiration of contaminated amniotic fluid. Late-onset disease, occurring between 1 week and 12 weeks of life, is thought to result from GBS contamination during passage through the birth canal. Both can present as sepsis, meningitis, bone and soft tissue infections, and pneumonia. In pregnant women, colonization with GBS is a risk factor for premature delivery, and ascending GBS spread can lead to chorioamnionitis and postpartum endometritis.5 Multilocus sequence typing (MLST),6 restriction digestion pattern,7 restriction fragment length pattern,8 and multilocus enzyme electrophoresis typing.9 MLST is an unambiguous sequence-based typing method that involves sequencing approximately 500-bp fragments of seven housekeeping genes. MLST is one of the most reliable tools for GBS typing, with sufficient discrimination for use in epidemiology and unambiguous characterization of isolates.6,10 There are few reports regarding serotyping, in particular, genotyping, of clinical GBS isolated in Taiwan. The aim of this study was to determine the serotype and genotype (by MLST) distributions in Taiwan and their correlations with clinical disease.

Methods

Collection of clinical isolates

Fifty clinical GBS isolates (33 from adults aged 18 years or older and 17 from infants aged 12 months or less) were collected during the period 2001–2004 at China Medical University Hospital, a medical center in central Taiwan with more than 2,000 beds. Isolates obtained from sterile sites, such as blood or body fluid (including cerebrospinal fluid, ascites, pleural effusion, and pericardial effusion), were indicated as invasive disease, and those from other sites were defined as noninvasive disease. Thirty-four strains were invasive (19 from blood, 13 from cerebrospinal fluid, and 2 from pleural and pericardial effusions). Sixteen were noninvasive (7 from cervical swabs of pregnant women, 6 from urine, and 3 from wound discharge).

Identification, serotyping, and MLST of GBS isolates

Isolates on blood agar were identified as GBS by the following criteria: narrow zone of beta-hemolytic colonies on 5% sheep blood agar plate, gram-positive cocci in pairs or short chains, negative reaction with catalase reagent, positive reaction with Christie/Atkins/Munch–Peterson test, and Lancefield grouping with serotype B antiserum. Capsular serotyping was classified by a grouping latex agglutination test (Phadebact Strep B Kit; Boule Diagnostic AB, Hudding, Sweden) and a coagglutination test (PhathoDx Strep Grouping Kit; Diagnostic Products Corp., Los Angeles, CA, USA).

MLST was performed as described by Jones et al.6 Initially, DNA from each isolate was extracted with a DNA isolation kit (Centurion Systems, Minneapolis, MN, USA). Seven housekeeping genes used for GBS characterization were amplified from DNA extracts by polymerase chain reaction, Taq polymerase (Applied Biosystems, Foster, CA, USA), and a polymerase chain reaction system (ABI 9700; Applied Biosystems). The seven loci were alcohol dehydrogenase, phenylalanine tRNA synthetase, amino acid transporter protein, glutamine synthetase, L-serine dehydratase, glucose kinase, and transketolase. The amplicons were sequenced by ABI Prism BigDye Termination Cycle Sequencing Ready reaction kit (The Perkin-Elmer Corporation, Foster City, CA, USA). The sequence of each locus was then uploaded to the GBS database at http://pubmlst.org/agalactiae/to obtain an allele designation. The combination of alleles at the seven loci was assigned to a unique sequence type (ST) for each isolate. The genetic relatedness of the strains was then assessed by cluster analysis with the unweighted pair group method with arithmetic average algorithm. The eBURST program (Department of Infectious Disease Epidemiology of Imperial College London, London) was used to group isolates into lineages or clonal complexes (CCs), which share six or seven identical alleles.11 The eBURST algorithm is implemented at http://eburst.mlst.net, and detailed user guide is available in this website.

Statistical analysis

Differences between groups were analyzed by the Fisher exact test, and multivariate analysis was performed with SAS software, version 9.1 (SAS Institute, Cary, NC, USA). A p value of 0.05 or less was considered statistically significant, and all tests of significance were two tailed.

Results

The mean age of the 33 adults was 55.8 years (range, 25–70 years). Among isolates obtained from nonpregnant adults, 73% (19 in 26 strains) were obtained from patients with malignancy, diabetes, or liver disease. Among the 17 infants, five episodes were early-onset disease and 12 episodes were late-onset disease. The characteristics of GBS isolates according to ST and serotype are listed in Table 1. The sequences of the seven MLST loci were determined for the 50 strains, and allelic profiles were assigned. The 50 isolates were resolved into 11 STs. A total of 90% of all isolates were composed of six major STs: ST1 (26%), ST12 (16%), ST19 (16%), ST17 (12%), ST23 (12%), and ST10 (8%). There was only one isolate each for five uncommon STs (ST3, ST16, ST106, ST139, and ST188). Among the six common STs, more frequent invasive
episodes were found for ST1 (10 of 13, 77%); ST12 (7 of 8, 87.5%); ST17 (6 of 6, 100%); and ST23 (5 of 6, 83%), compared with the mean invasive rate for all isolates (34 of 50, 68%). The most invasive ST1 and ST12 isolates (90% and 71%, respectively) were from adults; invasive isolates of ST17 and ST23 (100% and 80%, respectively) were always of neonatal origin.

The eBURST analysis revealed five major CCs and one single ST (ST61, n = 1) from 11 different STs. The most common one was CC1 (n = 14), followed by CC12 (n = 13), CC19 (n = 9), CC17 (n = 7), and CC23 (n = 6) (Table 1). CC1 consisted of ST1 and ST139; CC12 consisted of ST3, ST10, and ST12; CC19 consisted of ST19 and ST106; CC17 consisted of ST17 and ST188; and CC23 consisted of ST23. Most GBS isolates from CC1, CC19, CC17, and CC23 possessed a single ST (ST1, ST19, ST17, and ST23, respectively). Among adult isolates, there were significantly more from CC1 and CC12 compared with the remaining CCs (odds ratio = 12.44; p = 0.0003). Multivariate logistic analysis also resulted in an odds ratio of 29.63 and p < 0.0001. Stratification of adult and neonatal values resulted in an odds ratio of 5.83 for CC1 or CC12 as adult invasive isolates (p = 0.0570). Among neonates, there were significantly more isolates from CC17 and CC23 than from the remaining CCs (p < 0.0001).

Among various CCs, the most invasive isolates were found in CC1 (78.6%), CC17 (100%), and CC23 (83.3%). Most of the invasive cases of CC17 and CC23 (100% and 80%, respectively) were from neonates and those of CC1 (91%) were always from adults. Unweighted pair group method with arithmetic average was used to construct a dendrogram from the matrix of pairwise allelic differences between the 11 STs of the 50 isolates (Fig. 1).

Capsular serotype was determined for all isolates. The most common was serotype III (12 strains), followed by serotypes Ib (10 strains), V (9 strains), Ia (6 strains), IV (4 strains), II (2 strains), and VI (2 strains). The other five isolates were nontypable. No isolate of serotype VII or VIII was identified. Two of the six common STs corresponded to a single serotype: all ST12 and ST23 serotypes were of capsular serotype II.

Table 1. Characteristics of group B Streptococcus isolates according to ST and CC

<table>
<thead>
<tr>
<th>CC (numbers) and ST</th>
<th>Allelic profilea</th>
<th>Serotypes (no. of isolates)</th>
<th>No. of isolates (adult, neonatalb)</th>
<th>Invasive no. (adult, neonatalb)</th>
<th>Noninvasive no. (adult, neonatalb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1 (14)</td>
<td>1,1,2,1,1,1,2,2</td>
<td>V (8), VI (2), NT (2), Ib (1)</td>
<td>13 (12, 1)</td>
<td>10 (9, 1)</td>
<td>3 (3, 0)</td>
</tr>
<tr>
<td>139</td>
<td>1,1,4,1,1,2,2</td>
<td>V (1)</td>
<td>1 (1, 0)</td>
<td>1 (1, 0)</td>
<td>0</td>
</tr>
<tr>
<td>CC19 (9)</td>
<td>19</td>
<td>1,1,3,2,2,2,2</td>
<td>III (5), IV (3)</td>
<td>8 (6, 2)</td>
<td>3 (1, 2)</td>
</tr>
<tr>
<td>106</td>
<td>1,1,3,4,1,5,1</td>
<td>III (1)</td>
<td>1 (0, 1)</td>
<td>1 (0, 1)</td>
<td>0</td>
</tr>
<tr>
<td>CC12 (13)</td>
<td>12</td>
<td>10,1,4,1,3,3,2</td>
<td>Ib (8)</td>
<td>8 (6, 2)</td>
<td>7 (5, 2)</td>
</tr>
<tr>
<td>10</td>
<td>9,1,4,1,3,3,2</td>
<td>II (2), NT (2)</td>
<td>4 (4, 0)</td>
<td>0</td>
<td>4 (4, 0)</td>
</tr>
<tr>
<td>3</td>
<td>1,1,4,1,3,3,2</td>
<td>Ib (1)</td>
<td>1 (1, 0)</td>
<td>0</td>
<td>1 (1, 0)</td>
</tr>
<tr>
<td>CC17 (7)</td>
<td>17</td>
<td>2,1,1,2,1,1,1</td>
<td>III (5), IV (1)</td>
<td>6 (0, 6)</td>
<td>6 (0, 6)</td>
</tr>
<tr>
<td>188</td>
<td>2,1,1,2,1,1,2</td>
<td>III (1)</td>
<td>1 (0, 1)</td>
<td>1 (0, 1)</td>
<td>0</td>
</tr>
<tr>
<td>CC23 (6)</td>
<td>23</td>
<td>5,4,6,3,21,1,3</td>
<td>Ia (6)</td>
<td>6 (2, 4)</td>
<td>5 (1, 4)</td>
</tr>
<tr>
<td>Singleton (1)</td>
<td>61</td>
<td>13,1,6,4,1,1,1</td>
<td>NT (1)</td>
<td>1 (1, 0)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50 (33, 17)</td>
<td>34 (17, 17)</td>
<td>16 (16, 0)</td>
<td></td>
</tr>
</tbody>
</table>

a The allelic profiles are presented in the following order: adhP, pheS, atr, glnA, sdhA, glicK, and tkt.
b Up to 3 months old.

CC = clonal complex; NT = nontypable; ST = sequence type.

Figure 1. Unweighted pair group method with arithmetic average dendrogram showing the genetic relationship among the 11 sequence typings (ST) of 50 isolates. The allelic profile of each ST is shown in parentheses. eBURST analysis identified five clonal complexes (CCs) by sharing six or seven identical alleles. The ancestral genotype of the four groups (1, 12, 19, and 17).
serotypes Ib and Ia, respectively. The invasive rates among serotypes with more than five isolates (serotypes Ia, Ib, III, and V) were 83%, 80%, 75%, and 88%, respectively, more than the average invasive rate of all isolates (68%). Most of the invasive cases among serotypes Ib and V (62.5% and 100%, respectively) were from adults; invasive isolates of serotypes Ia and III (80% and 89%, respectively) were always of neonatal origin. Most of the serotype isolates Ia, Ib, and V (n > 2) were each confined to single CCs: 100% of serotype Ia GBS was in CC23, 90% of serotype Ib GBS was in CC12, and 100% of serotype V GBS was in CC1. All isolates of serotype Ia corresponded to one ST (ST23), but other serotypes with more than two isolates corresponded to two to four STs.

Discussion

GBS infection is an important cause of neonatal disease, and the use of antibiotic prophylaxis after the onset of labor or rupture of the membranes is highly effective, especially for high-risk groups, such as those with GBS colonization, preterm labor, or membrane ruptures. In adults, invasive GBS disease has increased, especially among those with underlying disease, such as malignancy, diabetes, or liver disease. In the present study, 43 of the 50 isolates were from neonates (n = 17), cervical swabs of pregnant women (n = 7), and adults with underlying disease (n = 19). This is the first report of MLST of invasive GBS in Taiwan. In the present study, the common STs were ST1 (26%), ST12 (16%), ST17 (12%), and ST23 (12%). These five STs are similar to those reported in previous studies, but there were differences in order.6,12 In respect to the invasion of several factors, such as the expression of adhesins and other virulence factors or host factors.13 For example, GBS in CC17 with serotype III, corresponding to CC17, and in CC1, most strains were from invasive neonatal diseases, similar to those of other regions, where most ST17 strains were invasive in neonates.6,12 Similarity was also found in ST19 strains, which were distributed in adults and neonates.13 However, in the present study, geographic differences were found for ST23 and ST1, with most arising from neonates and adults, respectively.6,13

The capsular polysaccharide of GBS is a major virulence factor for invasive disease, which is divided into nine serotypes (Ia, Ib, II–VIII).14 The capsular capsule of GBS is determined by genes in the CPS locus, which encode enzymes for polysaccharide synthesis.15,16 No isolate of serotype VII or VIII was found among our strains. The most common and most invasive strain was serotype III (24%), as was reported in other studies in Taiwan and in other countries.6,13,17 Strains of serotype III were equally divided into ST17 and ST19 (or CC17 and CC19). Most isolates of serotypes Ia, Ib, and V corresponded to ST23, ST12, and ST1 (or CC23, CC12, and CC1), respectively, and most strains of ST1, ST17, ST19, and ST23 expressed capsular serotypes V, III, and Ia, respectively. These distributions were similar to those of other reports.6,12,13,18 Serotype V GBS emerged in the 1990s and has become important in terms of clinical GBS disease, especially in nonpregnant adults.1,19,20 Serotype V of GBS is suspected to have undergone clonal spread in several countries because all isolates belong to CC1 in our study and in other studies.12,13 Most invasive diseases (septicaemia or meningitis) in neonates are caused by serotype III GBS (47%),13,17,18 followed by serotype la (23.5%). This result corresponds to the most prevalent maternal colonization serotypes III and Ia, but not V, in a previous study.21 Most GBS invasive strains in adults are serotypes la (23–31%), Ib (6–13%), II (8–13%), III (19–21%), and V (24–31%), according to previous reports, but those among our present isolates were serotypes V (47%) and Ib (29.4%).1,19 The transition of invasive strains from serotype III in neonates to serotype V or other in adults might be driven by the host immune response or allelic exchange by horizontal transfer or recombination.13 In addition, several epidemiologic investigations have shown changes of GBS serotype distribution over time and in different countries.1,13,19,20 This might reflect differences in serotype fitness, herd immunity changes, or the spread of antibiotic-resistant clones, and is important with respect to vaccine development.13,14 The shift of clinical serotype distribution to nonvaccine types in response to the introduction of pneumococcal vaccination of susceptible populations has been well documented.22

Overall, each serotype usually contains several different lineages, and each lineage is also composed of several serotypes. This indicates that serotype alone is not sufficient for phylogenetic lineage differentiation and that horizontal transfer of CPS genes, resulting in serotype switches, occurs within the same lineages.12,13 In addition, GBS in the same ST but with different serotypes could be genetically divergent.13 This could explain the divergent relations between serotypes and clinical presentations among different CCs. In CC17, most isolates were serotype III and from invasive neonatal diseases. In CC1, most strains were from invasive adult conditions but distributed in different serotypes. In our present study, isolates from adults were significantly from CC1 or CC12, all isolates of CC17 were from neonates, and CC19 and CC23 contained both adult and neonatal isolates. There was a trend for isolates of CC1 or CC12 to become more invasive in adult patients, but this did not reach statistical significance (p = 0.057), which might be because of fewer case numbers. These findings suggest that CC19 and CC23 are successful clones in both adults and neonates, whereas CC1 and CC12, and CC17 appear to be adapted to adults and neonates, respectively. These variations might reflect differences in several factors, such as the expression of adhesins and other virulence factors or host factors.13 For example, GBS in CC17 with serotype III, corresponding to restriction digest pattern analysis III-3,6 has been confirmed to have a higher production of sialic acid and possesses SPB1, which encodes a protein involved in epithelial cell invasion.24 This strain has also been demonstrated to be more virulent than other serotype III GBS strains.25 However, information regarding virulence among different CCs is presently inadequate for the clarification of various clinical presentations and requires further study.
There were several limitations in our study. First, the collected strain number was relatively small. Second, only one phenotypic typing (serotyping) and genotyping (MLST) were applied. Despite the small number of isolates in the present study, these results are the first regarding GBS genotyping in Taiwan. Similar results between our study and other reports indicate that relatively small numbers of GBS lineages are responsible for human disease or colonization. However, some differences between our results and those of others were found. These differences might arise from geographic variance or inadequate GBS study number.

Further comparative genetic studies of clinical significance will be essential for the elucidation of host tropism and evolution of GBS isolates, as well as for vaccine development.

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References