ORIGINAL ARTICLE

Serotypes, surface proteins, and clinical syndromes of invasive Group B streptococcal infections in northern Taiwan, 1998–2009

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Background: The incidence of invasive Group B streptococcal (GBS) infections is increasing in the elderly and immunocompromised adults in many countries worldwide. There are, however, few reports regarding the current status of the infection in northern Taiwan. This study investigated retrospectively the molecular epidemiology and clinical syndromes of the invasive GBS diseases in a tertiary care hospital in northern Taiwan over the past decade.

Methods: One hundred twenty episodes of invasive GBS disease were recorded at Cathay General Hospital, a tertiary care, teaching hospital in northern Taiwan, from January 1998 to June 2009. Clinical information was acquired from medical records. Capsular serotypes and alpha family of surface proteins were genotyped with multiplex and specific polymerase chain reaction.

Results: Of all episodes, 58.3% was found in the elderly (age ≥ 65), 36.1% in nonpregnant women and young adults (age 18–64), and 5.9% in the neonates (0–90 days). Case-fatality rate was 6.7%. Eighty-three (69%) of the invasive isolates were available for genotyping. In sharp contrast to the studies in southern Taiwan (1991–2004), Type Ib (26.5%) was the most frequent invasive isolate, followed by V (22.9%), III (18.1%), VI (12%), Ia (10.8%), II (6%), VIII (2.4%), and nontypable strain (1.2%). In particular, Serotype VI, which had been rarely implicated in invasive infection, emerged as a significant pathogen. A significant trend of increase in incidence

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was observed for the infection ($p < 0.0001$), with concurrent increase of cases in the elderly and of Serotype Ib and VI. There was significant association with young adults of Type II and III and chronic skin conditions and older adults with Type Ia and V and chronic cardiovascular diseases. Type V was closely associated with skin and soft tissue infection. Recurrent episodes (10%) occurred most often in patients with concomitant malignancy, with an average of 314 days for recurrence.

**Conclusions:** The incidence of GBS invasive infection among nonpregnant women and adults is rising in northern Taiwan, particularly in the elderly caused by Serotype Ib and VI. Population-based surveillance program should be implanted for assessment of the disease burden to the susceptible adult population.

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**Introduction**

Group B streptococci (GBS), or referred to as *Streptococcus agalactiae*, is the leading cause of neonatal pneumonia, septicemia, and meningitis and responsible for significant morbidity of vulnerable adult populations. Commonly colonized along the genitourinary tracts, the pathogens either gain access to the fetus by transcending across placenta and infecting amniotic fluid and fetus alike, or to a lesser extent, are acquired during vaginal delivery after aspiration of contaminated vaginal secretions. As a result of perinatal antimicrobial prophylaxis, a significant trend towards reduction in the incidence of neonatal GBS sepsis was seen over the past decade. Consequently, the burden of invasive GBS diseases has gradually shifted to adult population, in particular those older with chronic underlying diseases.

Multiple factors in the host and GBS have been shown to contribute to the development of systemic infection. Comorbidity, such as chronic cardiac disease/congestive heart failure, diabetes mellitus (DM), malignancy, gastrointestinal and liver diseases, and bedridden or residence in a nursing home were among those commonly seen in patients with invasive GBS infection. For the pathogen, the antiphagocytic capsules around the cocci are one of the most important virulence factors contributing to the invasiveness. At least nine different serotypes are identified, that is, Ia, Ib, and II—VIII; among them, Type III predominates among the neonatal infection, of which a recently identified surface serine-rich repeat protein (Srr-2) was implicated in the high virulence of the serotype. In contrast to the neonatal infection, specific virulence inherent in the polysaccharide capsule that distinguishes invasive serotypes from colonizers in the adult population remains largely unknown. Nevertheless, the capsular serotype distribution of invasive strains is not uniform and reflects the frequency of colonizing strains, which further is subject to geographical as well as temporal variation.

From the perspective of vaccination, it is important to institute programs for continual, population-based surveillance of GBS infection to include appropriate capsular polysaccharide (CPS) in vaccine.

The aim of the present study was to analyze GBS invasive infection retrospectively of the patients admitted to our hospital during the past decade (1998–2009). By comparing our results with those from southern Taiwan (1991–2004), we would see the trend of the systemic infection by GBS in Taiwan and possible clinical implication for the management of the infected patients.

**Methods**

**Study population and definitions**

Cathay General Hospital, an 800-bed, tertiary care, teaching hospital in northern Taiwan, has averaged 27,000 patients admission per year. From January 1998 to January 2009, 120 episodes of invasive GBS infections, defined as infection accompanied by isolation of the bacteria from blood or cerebrovascular fluid, were identified from laboratory records. Infection by GBS isolated within 2 weeks from the same patient was arbitrarily defined as one episode and were considered separate if occurred 2 weeks apart. Clinical information, including patients’ demographic data, clinical syndromes, comorbid diseases, underlying conditions, antimicrobial therapy and treatment duration, and mortality, were obtained directly from medical records. Death was attributed to the infection if the affected patient died within 7 days after the first day of culture-proved GBS infection. Cases were classified as early-onset (0–13 days) or late-onset (14–90 days) neonatal diseases, younger adult without pregnancy ($\geq 18–64$ years), and elderly ($\geq 65$ years). Although pregnant women with GBS infection are important subjects to study, there was no invasive GBS disease found in pregnant women during this study period.

**GBS strains**

The GBS strains preserved at $–80 ^\circ$C were recovered by resuspension of frozen stocks in Brain-Heart infusion broth and plating on 5% sheep blood agar, which were then incubated for 24 hours. Recovered strains were confirmed as GBS by Gram stain (positive cocci in chains), narrow zone of beta hemolysis, negative catalase, positive CAMP test, and the presence of Group B Lancefield antigen. Antimicrobial susceptibility for penicillin, vancomycin, and erythromycin was assessed by using the Kirby-Bauer disc diffusion test, according to standards by CLSI M100-S19.
Genotyping of GBS serotypes and alpha family of surface proteins

The CPS serotypes of the recovered GBS strains were characterized by genotyping of CPS synthesis gene clusters (cps) with polymerase chain reactions (PCRs) as described previously. In brief, GBS serotypes were first identified with PCR-based restriction fragment length polymorphism (PCR-RFLP) using primers flanking cpsG and cpsL for Sero-type Ia, Ib, II–VII and cpsR to cpsL for Type VIII. Serotypes characterized in this way were reconfirmed with a pair of primers unique for each serotypes. Genes for alpha family of surface proteins, including bca, epsilon/alpha1, alpha2, alpha3, alpha4, and rib were identified as described.

Statistical analysis

Data were collected and analyzed using Windows Office Excel and SPSS software version 16 (SPSS Inc., Chicago, IL, USA). Changes in incidence over time were analyzed by chi-squared test for trend. Correlations between nominal variables were assessed for significance by chi-squared test or Fisher’s exact test. A p value of 0.05 or less was considered statistically significant.

Result

During the period of January 1998 to June 2009, 108 patients with 120 episodes of invasive GBS disease were recorded. Seventy (58.3%), 43(36.1%), and 7(5.9%) of these episodes were found in the elderly (≥ 65 years old, range 65–93 years old), nonpregnant women and young adults (< 65 years old, range 22–64 years old), and the neonates (0–90 days, range 1–33 days), respectively. None of the patients were found between ages 3 months to 18 years. Female patients seemed, yet statistically insignificant, more prevalent in the elderly group than in the young adults group (57.8% vs. 41.2%, p = 0.12) (Table 1).

The incidence of invasive GBS diseases increased from 0.2 episodes per 1,000 admissions in 1998 to 0.9 episodes per 1,000 admissions in 2008 (p < 0.0001) (Fig. 1). Similarly, the percentage of GBS isolated from cases of positive blood culture also increased from 0.46% in 1998 to 1.40% in 2008 (p < 0.0001). Among three groups, only in the elderly group the incidence rate increased over time (p < 0.01) (Fig. 1).

Eight of the 120 invasive GBS episodes were fatal, accounting for a mortality rate of 6.7%. All GBS in these 120 episodes were susceptible to penicillin and vancomycin, and 47% were resistant to erythromycin.

Neonates and infants

Of the seven neonates with invasive GBS diseases, four (57%) cases were early onset (< 7 days after birth) and three (43%) were late onset (7–90 days after birth). Pneumonia was the most common manifestation (3, 43%), followed by meningitis (2, 28%), urosepsis (1, 14%) and bacteremia without identified source/primary bacteremia (1, 14%). All early-onset cases symptoms developed within 1 day, with the majority being of pneumonia (3, 75%). There was one preterm neonate with early-onset disease of...
primary bacteremia. Three late-onset neonatal diseases were identified, including two cases of meningitis, one pneumonia, and one urosepsis. The mortality rate in the neonatal group was 14.3% (1 term neonate with early-onset disease).

Adults

One hundred thirteen cases were identified in adults of 18 years or older (range 22–93 years). There were no cases identified of invasive GBS infections for pregnant women. Most clinical manifestations were skin and soft tissue infection (SSTI) (46, 40.7%), followed by primary bacteremia (28, 24.8%), osteomyelitis/septic arthritis (12, 10.6%), urinary tract infection (10, 8.8%), endocarditis (4, 3.5%), pneumonia (3, 2.7%), and polymicrobial bacteremia following lower gastrointestinal surgery (2, 1.8%). No association between clinical manifestations and age groups was found. However, chronic skin diseases were found predominant in the young adults group (18.4% vs. 0%, \( \text{p} < 0.001 \) by Fischer’s exact test), and patients in the elderly group were more likely to have chronic cardiovascular diseases (44.4% vs. 21.1%, \( \text{p} = 0.017 \)). Overall, chronic cardiovascular diseases are the most commonly associated comorbidity (35.6%) followed by malignancy and DM (each 30.7%). Genitourinary (11, 35.5%) and breast cancer (4, 12.9%) together account for nearly half of all cases with malignancy, with the majority associated with either SSTI (9/15) or urinary tract infection (2/15).

Serotypes and surface proteins

Of the 120 invasive episodes, 83 (69%) strains were recoverable for serotyping and surface protein characterization. Two strains of each Serotype Ia, Ib, and III were isolated from the neonates (86% recovery rate). The only fatal case in this age group was caused by Serotype III.

Among adult patients, Serotype II (5/5, \( \text{p} < 0.007 \)) and III (9/13, \( \text{p} = 0.014 \)) were closely associated with young adults, whereas Serotype Ia (6/7, \( \text{p} = 0.03 \)) and VI (9/10, \( \text{p} = 0.078 \)) were found chiefly in the elderly. With regard to clinical syndromes, Type V is highly associated with SSTI (10/18, 52.6%, \( \text{p} = 0.047 \)) (Table 2). Although Serotype VIII were only isolated from patients with osteomyelitis (2/2, \( \text{p} = 0.01 \)), the sheer case number precludes general conclusion made to the serotype. Two serotypes (Ib and VI) increased in incidence with time among annual positive blood cultures (\( \text{p} < 0.001 \) and \( \text{p} = 0.012 \), respectively). In particular, the two serotypes together accounted for nearly half of all recoverable isolated strains each year (Fig. 2), suggesting the trend for invasive GBS infection was attributable for most part to the emergence of these two specific serotypes.

To gain further insights into the population structure of the invasive GBS strains, we characterized bacterial surface proteins of the Alp family, which were encoded by \( \text{bca}, \text{alpha1/epsilon}, \text{alpha2}, \text{alpha3}, \text{alpha4}, \) and \( \text{rib} \) genes. It has been shown that the Alp family proteins are capable of eliciting host protective antibodies; therefore, information

Table 2  Distribution of Group B streptococcal capsular serotypes by clinical syndromes

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>Ia (%)</th>
<th>Ib (%)</th>
<th>II (%)</th>
<th>III (%)</th>
<th>V (%)</th>
<th>VI (%)</th>
<th>VIII (%)</th>
<th>NT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary bacteremia (n = 18)</td>
<td>4 (22.2)</td>
<td>6 (33.3)</td>
<td>2 (11.1)</td>
<td>3 (16.7)</td>
<td>3 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SSTI (n = 28)</td>
<td>2 (7.1)</td>
<td>6 (21.4)</td>
<td>2 (7.1)</td>
<td>4 (14.3)</td>
<td>10 (35.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Osteomyelitis/septic arthritis (n = 9)</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td>1 (11.1)</td>
<td>1 (11.1)</td>
<td>2 (22.2)</td>
<td>1 (11.1)</td>
<td>2 (22.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pneumonia (n = 6)</td>
<td>3 (50.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>UTI (n = 9)</td>
<td>0 (0)</td>
<td>4 (44.4)</td>
<td>0 (0)</td>
<td>3 (33.3)</td>
<td>2 (22.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Meningitis (n = 1)</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</tr>
</tbody>
</table>

<sup>a</sup> \( \text{p} < 0.05 \).

SSTI = skin and soft tissue infection; UTI = urinary tract infection.

Figure 1. Incidence of invasive Group B streptococcal disease among infants (0–90 days), young adults (< 65 years), and older adults (≥ 65 years).
for the distribution of these proteins among invasive GBS strains is as important as that for the immunogenic capsular antigens in vaccine development. Most surface protein genes in our isolates were bca (47%), followed by alpha3 (20%), rib (20%) and alpha1/epsilon (12%). Neither alpha2 nor alpha4 was found in our isolates. The rib gene was found more frequently in young adults (p = 0.006). Five major serovariants, including Ib/bca (26.5%), V/alpha3 (18.1%), III/rib (14.5%), VI/bca (10.8%), and Ia/alpha1 (8.4%), represented 78.3% of all isolates. Because both Serotypes Ib and VI are highly associated with the presence of bca (22/22 and 9/10, respectively), a trend for bca to increase with time was also observed (p = 0.001).

Eighteen of the 120 episodes were distributed among six patients who had total 12 recurrent invasive GBS diseases, with average interval of 314 days (range 49–875 days) for recurrence. Most of these patients (5/6, 83%) had malignancy. In particular, four patients with recurrent infection manifested SSTI for the first and subsequent recurrent episodes. Among five strains recovered from the 12 recurrent episodes, 3 shared the same capsular serotype and surface protein genes (i.e. same serovariants) with those isolated from the antecedent infection. The intervals for disease recurrence by these three strains were all less than 1 year (113, 337, and 347 days). In contrast, the other two strains of different serovariants from their respective predecessors were isolated more than 1 year away from antecedent episodes (411 and 716 days).

Discussion

Our study analyzing all GBS invasive infections in a medical center from 1998 to 2009 revealed several important facts. First, the incidence of the infection increased among patients admitted for in-hospital care, and the trend is mainly because of the increased frequency of the infection in the elderly (≥ 65 years). Second, compared with the results from studies in southern Taiwan (1991–2004), our data demonstrated a dramatic increase in the frequency of isolation of Serotype Ib and VI, both of which paralleled the general trend of the invasive diseases and concurrent decrease of Serotype III and Ia among the invasive isolates. Third, comorbid conditions and strain serotypes were distributed unevenly between young (< 65 years) and old (≥ 65 years) adults. Chronic skin diseases and Serotype II and III were found predominantly in the young adults, and chronic cardiovascular diseases and Serotype Ia and VI were found in the elderly. Fourth, Serotype V is closely associated with clinical manifestations of SSTI. Finally, recurrent episodes of invasive infection often presented as SSTI in patients with malignancy by strains of the same serovariants as preceding ones.

Increases in the incidence of invasive GBS disease in elderly adults have been observed worldwide, including Spain (1985–1994), the United States (1999–2005), and Norway (1996–2006), with up to fourfold increase over two decades (review in Refs.2,14). The increase in disease burden to the elderly may be a direct result of aging population with consequent immune senescence impairing cell-mediated immunity. In addition, several known comorbid conditions, which are predominantly associated with the older adults, are conducive to the acquisition of the infection as the population become older.2 Our retrospective analysis, albeit sampled from a medical center, revealed comparable trend in the disease incidence and characteristics of patients’ underlying conditions and clinical manifestations. This suggests that the invasive infection caused by GBS is an increasing threat to the adult population in Taiwan as are in other countries, especially to the elderly with comorbid conditions, such as malignancy, DM, cardiac, GI, and liver diseases.15

Prevalence of GBS capsular serotypes, on the other hand, differs in several aspects from previous studies in southern Taiwan (1991–2004). In contrast to the predominance of Serotypes III and V, which together accounted for more than 60% of all isolated strains in their studies, Ib (26%) and V (24.7%) are the two most frequently isolated strains in our study. This remarkable change in serotype distribution may be explained in part by the fact that most of our cases came from the adult population (94%), as Serotype III is known to be dominant in the neonatal cases.16 Differences in prevalence of serotypes between geographically distinct areas, that is, south versus north Taiwan, could lead to the discrepancy in serotype distribution. Of all, increasing incidence of Type Ib (Fig. 2) appeared to be the most important contributing factor. Change in serotype distributions over time is not uncommon.Persson et al.3 observed a twofold increase in Serotype V from the period of 1988–1991 to 1998–2001 in
west Sweden among strains isolated from patients of all age, with concurrent decrease in Serotype Ib for the adult cases. Our result reinforces the importance of regional dynamic change in serotype distribution, and timely active surveillance is vital to formulating effective vaccines for the population within the areas.

The emergence of Serotype VI, especially among those isolated after year 2006, is alarming. Type VI in general is not a common serotype of all CPS antigens. Prior study in southern Taiwan between 1994 and 2004 showed only 2.6% of Type VI in 156 strains. In sharp contrast, however, Serotype VI, along with VIII, were overwhelmingly represented by vaginal colonizing strains isolated from healthy pregnant Japanese women (24.7% and 35.6%, respectively). Given the geographical proximity and prosperous mutual tourism, the emerging of Type VI in our study may reflect a direct spread of GBS from Japan to Taiwan. Alternatively and more worrisome, such a change in serotype distribution beacons clonal expansion of virulent strains carrying the capsular antigen. In support of this, 90% (9/10) of all isolated Type VI carried the surface protein gene bca, suggesting some degree of relatedness among these strains. Further genome analysis of these strains to delineate the extent of clonality, in relation to those from Japan and Taiwan, should provide answers to these questions. The finding of Serotype V in association with SSTI is, to our knowledge, the first report linking Type V to a specific clinical syndrome. Except in the neonates, in whom Type III is associated with neonatal late-onset meningitis, there have been few reports for adult patients describing association of serotypes with particular syndromes. On the other hand, different serotypes seem to vary in their immunogenicity during naturally acquired subclinical infection. For example, serotype-specific IgG against Type VIII was at higher level in healthy colonized women than those against Ia, Ib, II, and III. In contrast, the level of Type V antibody in healthy colonized elderly persons was the lowest among all measured type-specific antibodies. The variation in immunogenicity was not observed in vaccinated volunteers, suggesting that different serotypes may be equally potent to generate host adaptive immunity. The ability to evade host innate immunity, however, may not be equal among strains of different serotypes during natural infection. Thus, Serotype V may be more susceptible to macrophage bactericidal functions and thus prone to localized inflammation, making it difficult to cause systemic spread and effectively generate adaptive antibody response. Metastatic infections remote to the primary colonized foci, which manifest as osteomyelitis and endocarditis, signify failure in local innate immunity and therefore are characteristic manifestations by more invasive serotypes. In our study, none of the patients with bacteremic cellulitis caused by Serotype V (10/28, 36%) had chronic skin conditions, indicating that SSTI was more likely because of virulent factors inherent in Serotype V strains rather than gross defects over skin or mucus, which were prone to local infection and/or prolonged colonization. In spite of the significant association, however, the invasive isolates in our study were restricted to cases from a single community, and we cannot exclude potential interference by geographical clustering, which would be eliminated by population-based active surveillance for assessing the prevalence of GBS colonization as well as invasive infection.

Recurrence of GBS invasive infection is not uncommon. In most cases reported, recurrent episodes were relapses from deep foci seeded by antecedent bacteremia or prolonged colonizing strains because of hosts’ underlying conditions or related treatments. The interval between the first and recurrent episodes was shorter if caused by the same strain (i.e. relapse) than by different strains (i.e. reinfection), with means of 14 and 43 weeks, respectively. Relapsing episodes often manifested osteomyelitis or endocarditis rather than SSTI and were caused by inadequate antimicrobial therapy for antecedent bacteremia. On the contrary, our recurrent cases manifested mostly SSTI and if occurring close to the respective preceding infections, they were caused by strains of the same serovariants. Given that most patients with recurrent infection have concomitant malignancies, it is likely that impaired host immune system fail to generate adequate protection against reinfection by strains of identical serovariants. Alternatively, prolonged colonization on the patients or their house contact may increase the chance of disease relapse. Further genome analysis of these strains should help clarify the nature of the recurrent infection (i.e. reinfection vs. relapse) and assist our management for preventing recurrent infection to immunocompromised population.

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