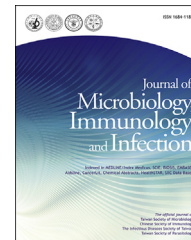




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Original Article

Serotype distribution and clinical correlation of *Streptococcus agalactiae* causing invasive disease in infants and children in Taiwan



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Abstract *Background:* *Streptococcus agalactiae*, or group B *Streptococcus* (GBS), remains to be one of the leading pathogens causing invasive infections in infants.

Methods: The clinical GBS isolates from sterile sites of patients younger than 18 years old were collected from October 1998 to December 2014 in two hospitals in Taiwan. Medical records were retrospectively reviewed. Every isolate was serotyped with a multiplex PCR assay. Multi-locus sequence typing (MLST) was performed in representative isolates of different serotypes. A total of 205 GBS isolates were collected from 181 patients with 182 infection episodes.

Results: Serotype Ia was the most common in patients less than 72 h old, whereas III the most common in patients older than 72 h. In early-onset disease (0–6 days), Ia and III each caused 27.5% of the infection, followed by Ib (14.5%). In late-onset disease (7–89 days), serotype III predominated (75.3%), followed by Ia (10.1%) and Ib (6.8%). Thirty-one episodes (17%) were complicated with culture-confirmed meningitis. We compared serotype Ia and III patients, and found that serotype Ia patients were significantly younger (median age, 3 days), had more

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perinatal maternal fever and higher mortality. ST17 and ST19 were exclusively found in serotype III, while ST23 and ST24 comprised of 85% of serotype Ia.

Conclusion: In Taiwan, serotypes Ia and III are the most common cause for early-onset and late-onset neonatal GBS infections, respectively. Some differences in the clinical features of invasive GBS infections caused by serotype Ia and III were observed.

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Background

Streptococcus agalactiae, or group B *Streptococcus* (GBS), is an important pathogen causing invasive infections in neonates and infants. In 1970s, the fatality rates could be as high as 50%.¹ After the introduction of universal screening and intrapartum antibiotic prophylaxis (IAP) in the United States, there was a striking decline of the disease incidence.² GBS infections in young infants are usually divided into early-onset disease (EOD, 0–6 days old) and late-onset disease (LOD, 7–89 days old). While there was a satisfactory reduction in EOD, the disease burden of LOD remained substantially high.^{3,4} GBS is still among the leading pathogens causing morbidity and mortality in infants in many countries, including Taiwan.^{5–8}

There are ten known serotypes of GBS: Ia, Ib, and II–IX. They have distinct capsular polysaccharide structure, an important virulence factor causing human diseases.^{9–11} Although neonatal GBS infection is closely related to the maternal genital colonization, previous studies have observed a very different distribution of serotypes and phylogenetic lineages between invasive neonatal disease and maternal colonization.^{9,12,13} A WHO-funded systematic review and meta-analysis reported that globally, 48.9% of invasive GBS disease was caused by serotype III in infants younger than 3 months. Serotype Ia accounted for 22.9%, Ib 7.0%, II 6.2%, and V 9.1%.¹⁴ However, the study included no data from Asia.

Previous studies of invasive GBS disease in infants are limited in case numbers, incomplete clinical information, or lacking microbiological data.^{15–17} In this study, we aimed to demonstrate the clinical correlation of different serotypes and genotypes of GBS derived from infants and children younger than 18 years old with invasive GBS infections.

Methods

Study population

Chang Gung Memorial Hospital at Linko (CGMH Linko) is a tertiary medical center in northern Taiwan, and Chia-Yi Christian Hospital a local hospital in southern Taiwan. All the clinical GBS isolates from sterile sites between October 1998 to December 2014 in CGMH Linko and June 2011 to December 2014 in Chia-Yi Christian Hospital were identified. The isolates derived from patients younger than 18 years old were collected and the medical records of these patients were retrospectively reviewed. Bacteremia is

defined as positive blood culture, and meningitis as positive cerebrospinal fluid (CSF) culture of GBS. We also identified the paired isolates from the same patient, and counted them as only one infection episode, in order to show a more reliable serotype distribution of GBS. The clinical information collected included age, symptoms, signs, laboratory data (including the susceptibility test of the isolate), antibiotics usage, accompanied diseases, length of stay, gestational age, birth weight, and maternal histories. This study was approved by the Institutional Review Board of CGMH Linko (103-0531B and 103-2479B).

Serotyping

The identification of GBS isolates was carried out in the Clinical Microbiology Laboratory of the two hospitals using standard tests of CAMP (Christie, Atkins and Munch-Petersen) test and latex agglutination. Every isolate was further serotyped with a multiplex PCR (polymerase chain reaction) assay, developed by Imperi et al.¹⁸ Three PCR primer sets were used for this examination. The first contained primer pairs specific for serotypes: Ia, Ib, II, III, and IV; the second contained primer pairs for serotypes: V, VI, VII, VIII and IX; and the third contained a primer pair for GBS-specific *dltS* gene, an internal positive control. The clinical GBS strains grown overnight on blood agar plates at 37 °C with 5% CO₂ supplement were applied for DNA extraction using Tissue & Cell Genomic DNA Purification Kit (Gene Mark, Taipei, Taiwan), followed by PCR examination. The PCR products were analyzed for the patterns of different serotypes using gel electrophoresis.

Multilocus sequence typing (MLST)

After we serotyped all the isolates, we randomly chose some of the isolates of each serotype to perform MLST. Twenty isolates were selected in each of the most common serotypes: Ia, Ib, and III. Twenty-three isolates were selected for the rest of the serotypes. An online database (<http://pubmlst.org/sagalactiae>) was used to assign alleles of the 7 loci and each isolate was defined by their sequence type (ST).¹⁹

Statistical analysis

In descriptive analysis, mean and standard deviation (SD), or median and interquartile range (IQR), were used to describe the central tendency and spread for continuous

variables when appropriate. Comparisons of the variables between groups were made using Chi-square test, independent t test, or Wilcoxon rank-sum test, when appropriate. For the analysis of survival time, Kaplan–Meier method was used. A log-rank test was used to compare the survival curves between different serotypes. All analyses were performed using SAS statistical software version 9.3. A p -value < 0.05 was considered statistically significant.

Results

Totally, 205 GBS clinical isolates fulfilling the inclusion criteria were identified. They were from 181 patients with 182 infection episodes. One patient had two separate episodes of GBS bacteremia, which occurred 14 days apart and were caused by different serotypes. All the other paired

isolates from the same patient were cultured less than 72 h apart and belonged to the same serotypes, thus each paired isolates were considered as one infection episode. Twenty-one of the paired isolates were from blood and CSF, one from blood and pleural effusion, and another one from blood and deep tissue culture. The details of the serotype distribution of the 182 infection episodes are listed in Table 1 and also in Fig. 1. Twenty-two patients died due to the infection, resulting in a 12.1% mortality rate. The mortality rate of patients younger than 3 months old was 13.6%.

Overall, serotype III caused 98 (53.9%) infection episodes, followed by Ia: 31 (17.0%), Ib: 19 (10.4%), VI: 10 (5.5%), V: 7 (3.9%), II: 6 (3.3%), VII: 1 (0.6%), and non-typeable: 10 (5.5%). However, at different age of disease onset, the serotype distribution varies. Serotype Ia is the most common in patients less than 72 h old, whereas III is the most common in

Table 1 Clinical diagnosis and distribution of GBS serotypes in different age groups in 182 episodes.

Groups	Diagnosis	Ia	Ib	II	III	IV	V	VI	VII	NT
Early-onset disease (0–6 days, $n = 68$)	Meningitis	1	0	0	1	0	0	0	0	0
	Bacteremia	17	6	4	17	0	6	5	1	5
	Both	1	3	0	1	0	0	0	0	0
	Others	0	0	1	0	0	0	0	0	0
Late-onset disease (7–89 days, $n = 88$)	Meningitis	0	0	0	2	0	0	0	0	1
	Bacteremia	6	6	1	51	0	0	1	0	2
	Both	3	1	0	13	0	0	0	0	1
	Others	0	0	0	1	0	0	0	0	0
Older infants/children (3 months to 18 years, $n = 21$)	Meningitis	0	0	0	0	0	0	0	0	1
	Bacteremia	1	2	0	12	0	0	3	0	0
	Both	1	1	0	0	0	0	0	0	0
	Others	1	0	0	0	0	1	1	0	0

The 177 of the total 182 episodes of infections are shown in meningitis and bacteremia, and the other 5 specimens are: ascites (serotype II, early-onset), hip joint pus (serotype III, late-onset), pleural effusion (serotype VI, children), and deep tissue cultures (serotype Ia, serotype V, children). NT: non-typeable.

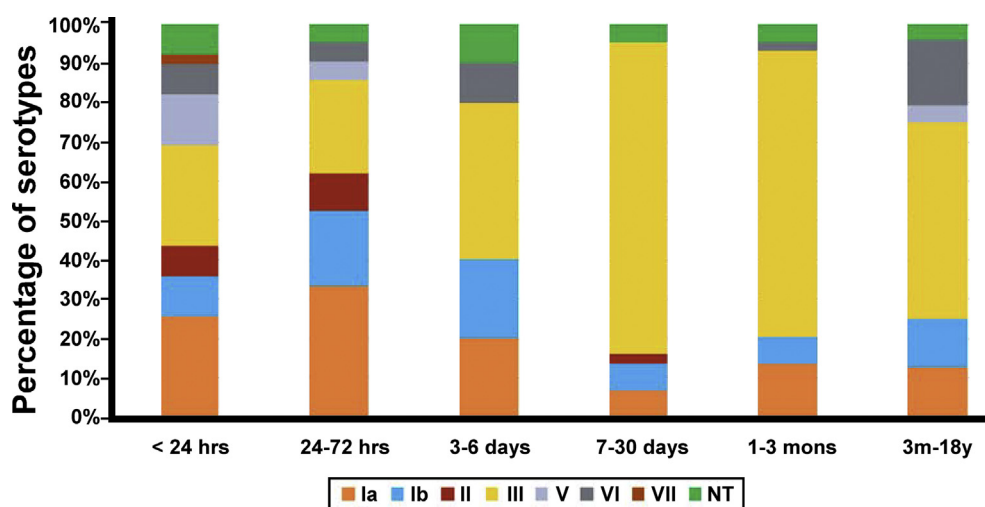


Figure 1. Percentage of serotypes among different age groups. <24 h: Ia (25.6%), Ib (10.3%), II (7.7%), III (25.6%), V (12.8%), VI (7.7%), VII (2.6%), NT (7.7%); 24–72 h: Ia (33.3%), Ib (19.0%), II (9.5%), III (23.8%), V (4.8%), VI (4.8%), NT (4.8%); 3–6 days: Ia (20%), Ib (20%), III (40%), VI (10%), NT (10%); 7–30 days: Ia (6.8%), Ib (6.8%), II (2.3%), III (79.5%), NT (4.5%); 1–3 months: Ia (13.6%), Ib (6.8%), III (72.7%), VI (2.3%), NT (4.5%); 3 months to 18 years: Ia (12.5%), Ib (12.5%), III (50%), V (4.2%), VI (16.7%), NT (4.2%). *NT: non-typeable.

patients older than 72 h old. Serotype distribution of different age groups is shown in Fig. 1. In early-onset disease (0–6 days), Ia and III each caused 27.54%, followed by Ib (14.5%). In late-onset disease (7–89 days), serotype III predominated (75.3%), followed by Ia (10.1%) and Ib (6.8%). There were 7 patients older than one year old, 4 of them were caused by serotype VI, the remaining 3 patients were each caused by serotype Ia, III, and V. The survival curve of patients with serotype III infections was significantly better than those with non-type III infections (Fig. 2).

Thirty-one of the 182 infection episodes (17.0%) were complicated with culture-confirmed meningitis. The serotypes of the CSF isolates are illustrated in Fig. 3. Eight of the meningitis patients were early-onset, 20 were late-onset, and 3 of them were older infants (range of age: 2–130 days). Thus 11.6% of early-onset disease and 22.5% of late-onset disease were complicated with meningitis. Three patients with meningitis died, giving a mortality rate of 9.7% in the meningitis group, which is lower than the overall 12.1% mortality.

Serotype Ia and serotype III caused 70.9% of the infections. We compared the clinical parameters of the two groups of patients (Table 2). In serotype Ia group, the median age was 3 days, much younger than serotype III group (25.5 days) ($p = 0.0018$). Fever was found at disease onset in 48.4% of serotype Ia and 62.2% in serotype III infections ($p = 0.1717$). Perinatal maternal fever was noted in 12.9% of serotype Ia and 1.0% of serotype III patients ($p = 0.0118$). The mortality rate in the serotype Ia group was 21.4%, markedly higher than the 4.6% mortality in the serotype III group ($p = 0.0125$).

The antibiotic susceptibility testing showed 100% susceptibility to ampicillin, second and third generation cephalosporins, and glycopeptides. Serotype III was more resistant to clindamycin, erythromycin, and chloramphenicol (Table 3).

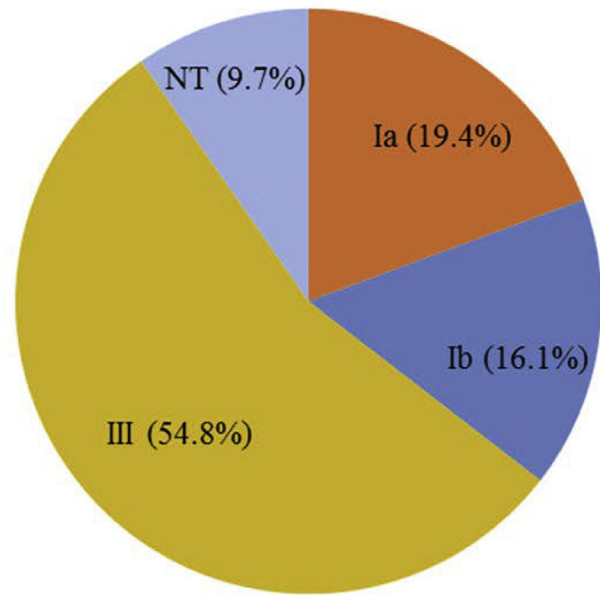


Figure 3. Serotypes causing meningitis. NT: non-typeable. Culture-confirmed meningitis: 31 patients. Serotypes: Ia = 6, Ib = 5, III = 17, NT = 3.

Among the 20 isolates in serotype Ia we performed MLST, 13 were ST23, 4 ST24, 1 ST144, and 2 remained undetermined. As to the 20 isolates in serotype Ib, 19 were ST12, and one ST43. The MLST of 17 serotype III isolates revealed a more diverse result; 7 were ST17, another 7 ST19, 1 ST1, 1 ST188, 1 ST456, and 3 remained undetermined. The results of MLST are demonstrated in Table 4. ST1 was detected in multiple serotypes, whereas ST12 was specifically in serotype Ib, ST17/ST19 in serotype III, and ST23/ST24 in serotype Ia.

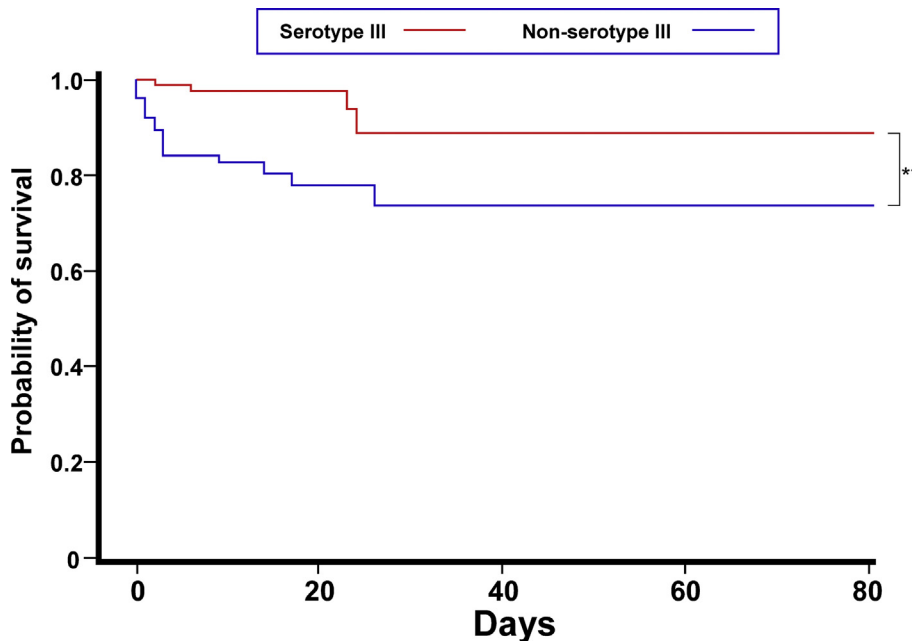


Figure 2. Kaplan–Meier plot of survival curves, stratified by serotype III ($n = 98$) and non-serotype III ($n = 84$). $**p = 0.0015$.

Table 2 Comparisons between serotype Ia and III.

Features	Serotype Ia	Serotype III	p Value
	n = 31	n = 98	
Age ^a (days)	3.0 (IQR 34)	25.5 (IQR 43)	0.0018*
Preterm (GA ^b < 37 weeks) (%)	14.8 (4/27)	22.8 (18/79)	0.5828
Low birth weight (<2500 g) (%)	18.5 (5/27)	15.5 (13/84)	0.7660
Birth via cesarean section (%)	20.0 (5/25)	33.3 (24/72)	0.2096
Fever presented (%)	48.4 (15/31)	62.2 (61/98)	0.1717
Perinatal maternal fever (%)	12.9 (4/31)	1.0 (1/98)	0.0118*
Respiratory distress (%)	19.4 (6/31)	14.3 (14/98)	0.4967
Seizure presented (%)	0 (0/31)	5.1 (5/98)	0.3362
Mortality (%)	21.4 (6/28)	4.5 (4/88)	0.0125*
White blood cell (WBC/ μ L)	9542.9 \pm 6841.4	10,051.2 \pm 7195.5	0.7432
Hemoglobin (g/dL)	12.6 \pm 3.1	11.9 \pm 2.3	0.2620
Platelet (μ L)	266,071 \pm 151,989	346,682 \pm 149,778	0.0152*
C-reactive protein (CRP, mg/L)	51.2 \pm 63.7	39.1 \pm 60.5	0.3725
Glucose (mg/dL)	84.4 \pm 26.2	125.6 \pm 61.2	0.0050*
Total antibiotics duration (days)	12.6 \pm 8.2	15.4 \pm 11.5	0.2397
Length of stay (days)	13.5 \pm 9.9	18.5 \pm 18.5	0.0562
Meningitis (%)	19.4 (6/31)	17 (17.4)	0.7991

^a Age presented as median (interquartile range, IQR).

^b Gestational age.

* $p < 0.05$.

Table 3 Antibiotic susceptibility of GBS serotypes.

Features	Ia	Ib	III	p Value
Ampicillin susceptible (%)	100	100	100	
Clindamycin susceptible (%)	64.7	15.4	31.4	0.01
2nd Cephalosporin susceptible (%)	100	100	100	
3rd Cephalosporin susceptible (%)	100	100	100	
Erythromycin susceptible (%)	88.2	30.8	22.4	<0.001
Penicillin susceptible (%)	92.9	100	100	0.025
Glycopeptide susceptible (%)	100	100	100	
Chloramphenicol susceptible (%)	100	100	54.5	0.039

2nd Cephalosporin refers to second generation cephalosporins. 3rd Cephalosporin refers to third generation cephalosporins. Serotype Ia, Ib, and III resulted in 81.3% of total infections.

Discussion

The study focused on invasive GBS disease in infants and children, providing useful clinical as well as molecular microbiological information of a large number of patients. We demonstrated a serotype distribution according to patients' onset age, to a detailed extent that has not been shown in previous studies. Overall, serotype III caused approximately half of the infections, but serotype Ia predominated in patients younger than 72 h old. A recent study in China showed that 85% of the invasive neonatal GBS

Table 4 Multilocus sequence typing (MLST) of 83 GBS isolates.

MLST	Serotype (No.)						
	Ia (20)	Ib (20)	II (6)	III (20)	V (6)	VI (10)	VII (1)
ST1	0	0	4	1	3	10	1
ST10	0	0	1	0	1	0	0
ST12	0	19	0	0	0	0	0
ST17	0	0	0	7	0	0	0
ST19	0	0	0	7	0	0	0
ST23	13	0	0	0	1	0	0
ST24	4	0	0	0	0	0	0
Others	1 (ST144)	1 (ST43)	1 (ST387)	2 (ST188, ST456)	0	0	0
UD	2	0	0	3	1	0	0

UD: undetermined.

infections were caused by serotype III.²⁰ According to another study in France,²¹ serotype III was predominant in both EOD and LOD, accounting for 57% and 80% of the infections, respectively. A report in South Africa revealed similar results, with serotype Ia being the most common in EOD (48.5%).⁷ We identified 7 patients older than one year old with invasive GBS disease, and four (58%) of them were caused by serotype VI. The serotype distribution of invasive GBS disease in patients one to 18 years old were rarely reported in the literature. Salloum et al. analyzed 75 isolates of invasive GBS disease in adults age 28–98 years old in France, and there was none with serotype VI.²²

In EOD, we found 88.4% of the patients had isolated bacteremia, 2.9% isolated meningitis, 7.3% both, and 1.5%

peritonitis, whereas in LOD, 76.4% of the patients had isolated bacteremia, 3.4% isolated meningitis, 20.2% both, and 1.1% arthritis. Thus 23.6% of LOD was complicated with meningitis, compared to 10.2% of EOD. In the above mentioned study in France,²¹ Joubrel et al. demonstrated a much higher percentage of meningitis in EOD (27%) and LOD (56%). However, Bauserman et al. reported a 98% bloodstream infection in EOD and 92% in LOD in US patients.²³ Only 7% of the LOD was complicated with meningitis.²³ Dangor et al. reported that in South Africa, 3% of EOD and 58.9% of LOD had culture-proved meningitis.⁷ Our data showed that meningitis was more common in LOD. Furthermore, our data was in agreement with previous studies that most of the meningitis was related to serotype III.^{24,25}

We were concerned about the differential clinical presentations of specific serotypes; thus we compared infections caused by the most common two serotypes, Ia and III. Although serotype III was more prevalent, serotype Ia caused a significantly higher mortality. This may be related to the younger age of the patients in serotype Ia disease. We also found that the patients with serotype Ia infections were associated with more maternal fever and lower platelet count and serum glucose level. Twenty percent of the patients in serotype Ia and 33.3% in serotype III diseases were delivered via Cesarean section (C/S). The C/S rate was rarely discussed in patients with GBS infections. However, the high C/S rate in both groups probably indicated a distressed fetal condition. In this study we also demonstrated a Kaplan–Meier plot, revealing that serotype III had a better probability of survival over non-serotype III infections. More studies may be needed before we could truly understand the reasons behind these findings.

GBS isolates remained susceptible to β -lactam antibiotics and glycopeptides, irrespective of the serotypes. This is compatible to studies from different regions of the world.^{17,21,26} Serotype III was relatively more resistant to clindamycin, erythromycin, and chloramphenicol. The higher antimicrobial resistance of GBS isolates was not associated with a higher mortality, probably because the empirical treatment of sepsis always included β -lactam antibiotics, which are effective to all GBS serotypes.

ST17 and ST19 were almost exclusively found in serotype III, compatible with other studies.^{21,27} Serotype III, especially the ST17 clone, was considered to be hyper-virulent and related to meningitis.^{9,28,29} The ability of ST17 to form a biofilm in acidic environment was considered one of the virulence factors.³⁰ However, in our study, patients with serotype III infections showed a lower mortality rate, despite a higher percentage of meningitis. The virulence factor of serotype Ia remains not fully understood. Nagano et al. hypothesized that the high sialic acid content in the capsular polysaccharides of serotype Ia and III might be related to their virulence.³¹ In a recent study from Portugal,³² serotype Ia (mainly ST23 and ST24) was the most common causing invasive infections in non-pregnant adults. In our data, 85% of the serotype Ia were ST23 and ST24, in consistent with previous studies.^{20,25}

A CRM₁₉₇-conjugated trivalent (Ia, Ib, III) GBS vaccine is under development.³³ If that vaccine were available, in Taiwan it might prevent 69.6% of early-onset disease, 92.2% of late-onset disease, and 91.3% of GBS meningitis, according to the present study.

The limitations of our study include the retrospective design and the limited study population from only one medical center and one regional hospital in Taiwan. The clinical information we could retrieve from the medical records depended largely on the judgment of the physicians in charge of the patients. We could not perform MLST in all the isolates, and this might lead to a less representative sequence typing results.

Conclusions

We found some clinical correlations between different serotypes and diseases caused. The information provided by this study is important to physicians for the prevention and treatment of GBS disease in infants and children.

Competing interests

The authors declare that they have no conflict interests.

Acknowledgments

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