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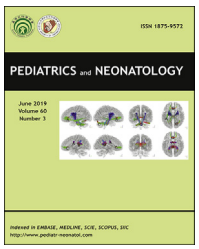

Original Article

Characterization of group B *Streptococcus* colonization in full-term and Late-Preterm neonates in Taiwan

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Key Words
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PFGE;
sequence type

Background: Group B streptococcus (GBS) infections can be life-threatening in newborns. This study aimed to analyze GBS carriage status and genotypic diversity in healthy neonates after implementation of intrapartum antibiotic prophylaxis (IAP) in Taiwan.
Methods: Newborns carrying GBS were identified from a screen of 500 newborns and followed up until their cultures turned negative. Their mothers' GBS screening data were reviewed. Molecular methods, including capsular serotyping, multilocus sequence typing and pulsed-field gel electrophoresis (PFGE), were used to analyze GBS isolates.
Results: GBS colonization was detected at either the nose or anus in 11 of 500 healthy neonates (2.2%). In this group of 11 neonates, 4 had GBS serotypes II and III for 4–6 months, 1 had serotype V for 2 months, 6 had serotypes Ia, II, V, and VI for less than 1 month, and 1 had 2 different serotypes (serotypes V and II) at different times. The most prevalent serotype was II (33.3%), followed by Ia (25.0%), III (16.7%), V (16.7%), and VI (8.3%). The main sequence type was ST1 (50.0%), followed by ST19 (16.7%), ST23 (8.3%), ST24 (8.3%), ST103 (8.3%), and ST 231 (8.3%). All isolates were grouped into 5 PFGE clusters F, G, J, X, and Y, and all were susceptible to β–lactam antimicrobial agents.

List of Abbreviations: GBS, Group B streptococcus; IAP, intrapartum antibiotic prophylaxis; PFGE, pulsed-field gel electrophoresis; PCR, polymerase chain reaction; MLST, multilocus sequence typing; ST, sequence type; CC, clonal complex.

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Conclusions: GBS was carried in 2.2% (11/500) healthy newborns and persisted for 6 months in 3 neonates. This study makes clearer our understanding of GBS colonization, serotype distribution, and genotype distribution in healthy neonates.

1. Introduction

Group B *Streptococcus* (GBS) is one of the most common pathogens causing meningitis, bacteremia, and pneumonia with high mortality rates in neonates, non-pregnant women, the elderly, and immunocompromised patients.[1] Not every baby who is born to a GBS-positive mother will be colonized or infected, but vertical transmission of GBS from mother to newborn during labor can result in life threatening infections.[2]

Neonatal GBS disease is divided into early-onset disease (occurring 0—6 days after birth) and late-onset disease (occurring 7—89 days after birth). Early-onset GBS septicemia is more commonly associated with pneumonia, which is believed due to aspiration of GBS during birth, while late-onset septicemia is more often accompanied by meningitis.[3,4] The risk factors of neonatal GBS infection include maternal GBS colonization, preterm labor, premature rupture of membrane, peripartum fever, a previous baby with GBS disease, and maternal GBS bacteriuria.[5]

The Center for Disease Control and Prevention (CDC) in the USA has recommended that all pregnant women be routinely screened for vaginal GBS between the 35th and 37th week of pregnancy.[6] The American Academy of Pediatrics has recommended that all women who have risk factors prior to being screened for GBS (for example, women who have preterm labor beginning prior to 37 completed weeks' gestation) should be treated with intravenous antimicrobial agents until their status of GBS is established.[7] Routine maternal screening and intrapartum antibiotic prophylaxis (IAP) have been very effective in preventing early-onset GBS disease. Given the significant burden of GBS disease, continued investigation of vertical transmission, virulence factors, risks of colonization, and also the genotypic diversity and antimicrobial susceptibility of GBS isolates is still needed. To date, no epidemiological data regarding the serotype distribution of GBS colonization in healthy neonates has been reported in Taiwan. Thus, we carried out a survey study of healthy, full-term neonates to determine GBS colonization status, and serotypes and genotypes of the colonizing isolates after implementation of IAP at a single medical center in Taiwan.

2. Materials and methods

2.1. Enrollment of healthy newborns

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (103-5109A3 and 106-1510C). In total, 500 newborns were enrolled from 2015 to 2016. GBS screening was conducted, according to policy, at 35—37 weeks of gestation in 337 of the mothers. The birth weights of the neonates were from 2200 to 4600 g.[8] The antimicrobial prophylaxis was provided to pregnant women with GBS colonization at least 4 h prior to delivery except in cases of sudden vaginal delivery or unknown carriage status without GBS testing, such as cesarean delivery.

2.2. GBS isolation

Nasal and rectal swabs were taken within 4—72 h after birth for all 500 neonates. The GBS-positive neonates were followed up monthly for GBS carriage until cultures turned negative. The swabs were first placed in LIM broth that contained nalidixic acid and colistin sulfate to inhibit the growth of Gram-negative bacteria and selectively enrich the growth of GBS.[9] Pure cultures of GBS were obtained by streaking the broth on GBS Detect™ agar plates (Hardy Diagnostics, Santa Maria, CA, USA) with incubation in a hypoxic chamber with 5% CO_2 at 37 °C overnight.

2.3. Serotyping by multiplex polymerase chain reaction (PCR)

The DNA of clinical GBS strains was extracted using a Tissue & Cell Genomic DNA Purification Kit (Gene Mark, Taipei, Taiwan). Then GBS isolates were serotyped for 10 serotypes using multiplex PCR.[10] The patterns of different serotypes were analyzed accordingly.

2.4. Multilocus sequence typing (MLST)

Sequence type (ST) of each isolate was determined by MLST. The allelic profile (sequence type [ST]) of each isolate was determined by matching each sequence to known allele sequences available at the online database (http://pubmlst.org/sagalactiae). The relationship between sequence types (STs) was drawn according to eBURST algorithm (http://eburst.mlst.net/).[11] Clonal complexes (CCs) of at least two STs having the same alleles at 6 of 7 loci were accordingly determined.

2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE profiling of GBS isolates was conducted according to the PulseNet Standardized Laboratory PFGE Protocol and using *Sma*I macrorestriction.[12] If *Sma*I could not digest GBS genomic DNA well, endonuclease *Xma*I (which recognizes the same nucleotide sequences as *Sma*I but cuts at different sites) was used instead (New England Biolabs,

Ipswich, MA, USA). The PFGE images were analyzed by the software BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). The UPGMA (unweighted pair-group method with arithmetic averages) was used to construct a dendrogram with similarity distance between any two PFGE images. A cluster with a Dice coefficient of ≥80% based on the result of the dendrogram was defined as a group or a cluster of two or more isolates.[13]

2.6. Antimicrobial susceptibility testing

The minimum inhibitory concentration of the antimicrobial agents (erythromycin, clindamycin, dalfopristin, daptomycin, ampicillin, cefotaxime and penicillin) to GBS was determined by the E-test method and the results were interpreted according to recommendations of the Clinical and Laboratory Standards Institute.[14]

2.7. Statistical analysis

Comparisons of the variables between groups were made using the Chi-square test. A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. GBS colonization in pregnant women and neonates

We screened for GBS in 337 of 500 mothers of healthy newborn babies during pregnancy. Eighty were GBS-positive (23.7%), while 257 were negative. The mothers of the remaining 163 babies did not receive GBS screening prior to delivery owing to cesarean section or birth at less than 37 weeks of gestational age (Table 1). Among the 500 neonates, only 11 were GBS-positive, indicating a 2.2% GBS colonization rate among full-term or late preterm healthy newborns. Four GBS-positive neonates were born to mothers who were GBS-positive during pregnancy; of these GBS-positive mothers, 3 had received IAP beginning less than 4 h before delivery and 1 beginning more than 4 h ($P = 0.031$ and 0.604, respectively, compared to GBS-negative mothers), suggesting that less than 4-h IAP could significantly increase neonatal GBS colonization ($P = 0.031$). The results also echoed the recommendation that IAP should be given at least 4 h before delivery for prevention of GBS infection. Additionally, neonatal GBS colonization was observed in 4 newborns born to GBS-negative women and 3 born to cesarean delivery women, suggesting that GBS from environmental sources could also colonize neonates.

3.2. Colonization sites

We identified 11 neonates who carried GBS at birth. Therefore, it is important to assess sites that could be potential portals of entry for GBS. Among the 11 GBS-positive neonates, 6 (1.2%) were positive at both the nose and anus and 5 were positive at the nose alone (2, 0.4%) or anus alone (3, 0.6%). The result indicated that both the respiratory tract and gastrointestinal tract could serve as portals of entry in neonates to initiate subsequent GBS infection.

3.3. Capsular serotypes and sequence types

Nasal or anal GBS colonization or both were followed up for 9 months in the 11 neonates. The GBS isolates were further analyzed by sequence typing and capsular serotyping. Serotype II and III strains were found to colonize neonates for 6 months, while serotypes Ia and VI colonized them for one month and serotype V strain for two months (Fig. 1A). No serotypes were detected at these sites after 9 months of age. In addition, there was a switch from serotype V (detected in the anus at birth and 1 month after birth) to serotype II (detected in the nose at two months after birth) in one case, and the two isolates from the same neonate shared the same sequence type ST1 (Fig. 1A).

The predominant sequence type in the 12 GBS isolates was ST1 (50.0%), followed by ST19 (16.7%), ST23 (8.3%), ST24 (8.3%), ST103 (8.3%), and ST231 (8.3%) (Table 2). The most prevalent serotype was II (41.7%), followed by Ia (25.0%), V (16.7%), III (8.3%), and VI (8.3%) (Fig. 1B; Table 2). ST1 was found in isolates with various serotypes, including II, V, and VI, and the sequence types of serotype Ia included ST23, ST24, and ST103. Using eBURST analysis, we identified four clonal complexes (CC1, CC19, CC23 and CC103). Of the four, CC1 contained ST1 and ST231, and CC23, ST23 and ST24.

3.4. PFGE dendrogram

To further verify the genetic relationship of the 12 GBS isolates, particularly the two serotype-switching GBS

Table 1 GBS colonization in both mother and neonate.

GBS screen results of mothers	GBS screen in neonates, N = 500		*P* value*
	Negative, N (%)	Positive, N (%)	
GBS-negative, N = 257	253 (98.4)	4 (1.6)	
GBS-positive, N = 80			
IAP < 4 h, N = 44	41 (93.2)	3 (6.8)	0.031
IAP ≧ 4 h, N = 36	35 (97.2)	1 (2.8)	0.604
No GBS test, N = 163			
(cesarean section and late-preterm birth)	160 (98.2)	3 (1.8)	1.0
Total, N = 500	489 (97.8)	11 (2.2%)	

* The *P* value of less than 0.05 is statistically significant. IAP: Intrapartum antimicrobial prophylaxis; NA: not available; N: number.

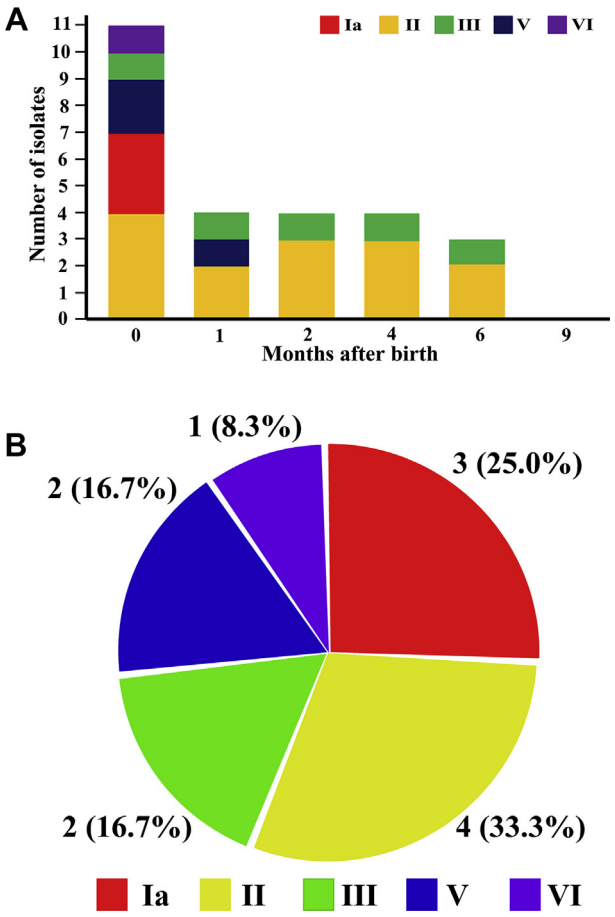

Figure 1 **(A)** The 12 GBS isolates carried by 11 neonates after 9 months of follow-up. Serotypes including Ia, II, III, V, and VI are indicated by individual colors. **(B)** Persistence of GBS in the nose and anus in 11 neonates. Serotypes, including Ia, II, III, V, and VI, are indicated by individual colors. A serotype switch from V to II in a carriage strain was observed during the period from the first to the second month.

isolates, PFGE was conducted. We classified the 12 isolates into two PFGE clusters X and Y with a Dice similarity coefficient of ≥80% and generated the UPGMA dendrogram based on these Dice coefficients (Fig. 2). The remaining PFGE patterns appeared to be different; however, three of them (clusters F, G, and J previously identified by Jiang's group) were the same.

Cluster Q contained two serotype II isolates that belonged to different sequence types (1 and 231). Cluster R comprised two strains sharing the same serotype V and sequence type ST1. Our examination of the serotype switch from V to II (as shown in Fig. 1A) found that the two GBS isolates (strains 294-10 and 294-22) shared the same sequence type ST1. However, their PFGE profiles with only 30% similarity suggested that they were completely different.

3.5. Antimicrobial susceptibility

In antimicrobial susceptibility, all 12 GBS isolates were susceptible to penicillin, ampicillin, and cefotaxime; however, 41.7% of the isolates, including 25.0% of CC1 isolates, 8.3% of CC19 isolates, and 8.3% of CC103 isolates, were resistant to clindamycin and erythromycin.

4. Discussion

This is the first study to determine GBS carriage rates in healthy neonates in Taiwan. We also carried out molecular characterization on 12 GBS isolates from 11 neonates. In this study, GBS was detected in 23.7% of mothers and 2.2% of neonates. Our rates were slightly different from those in a previously retrospective study involving 6 hospitals in Taiwan.[15] Among 4585 pregnant women in 2004–2005 and 63,367 newborns in 2002–2005 in Taiwan, maternal GBS colonization rate was around 20.0%, and the incidence of GBS disease in newborns and infants was 1.0%, with the mortality rate being 8.5% for early-onset disease and 3.8% for late-onset disease.[16] Our study showed that the rate of neonatal GBS colonization (or maternal carriage rate) has not changed in the past 10 years in Taiwan, even after nationwide adoption of GBS screening and IAP in 2012. Although the maternal carriage rate in Taiwan is close to that in the entire Asia–Pacific region (i.e., 19%), this rate actually varies among regions and countries, such as 8.0% in Korea, 8.2% in Japan, 7.1% in Beijing, China, and 7.1% in Hong Kong.[17–23]

Among the 10 serotypes of GBS, the five most common are Ia, Ib, II, III, and V,[12,13,24] accounting for 96 and

Table 2 Sequence types (ST) and serotypes of 12 GBS isolates from 11 neonates.[a]

CCs	STs	N (%)	Capsular Serotypes, N					Antimicrobial Resistance, %				
			Ia	II	III	V	VI	Cli	Ery	Pen	Amp	Ctx
CC1	ST1	6 (50.0)	0	3	0	2	1	25.0	25.0	0	0	0
	ST231	1 (8.3)	0	1	0	0	0	0	0	0	0	0
CC19	ST19	2 (16.7)	0	0	2	0	0	8.3	8.3	0	0	0
CC23	ST23	1 (8.3)	1	0	0	0	0	0	0	0	0	0
	ST24	1 (8.3)	1	0	0	0	0	0	0	0	0	0
CC103	ST103	1 (8.3)	1	0	0	0	0	8.3	8.3	0	0	0
Total N (%)		12 (100)	3 (25.0)	4 (33.3)	2 (16.7)	2 (16.7)	1 (8.3)	41.7	41.7	0	0	0

CCs: clonal complexes; STs: sequence types; N: number; uc: unclustered; Cli: clindamycin; Ery: erythromycin; Pen: penicillin; Amp: ampicillin; Ctx: cefotaxime.

[a] One neonate carried serotype V (ST1) at birth and 1 month after birth and serotype II (ST1) at 2 months after birth.

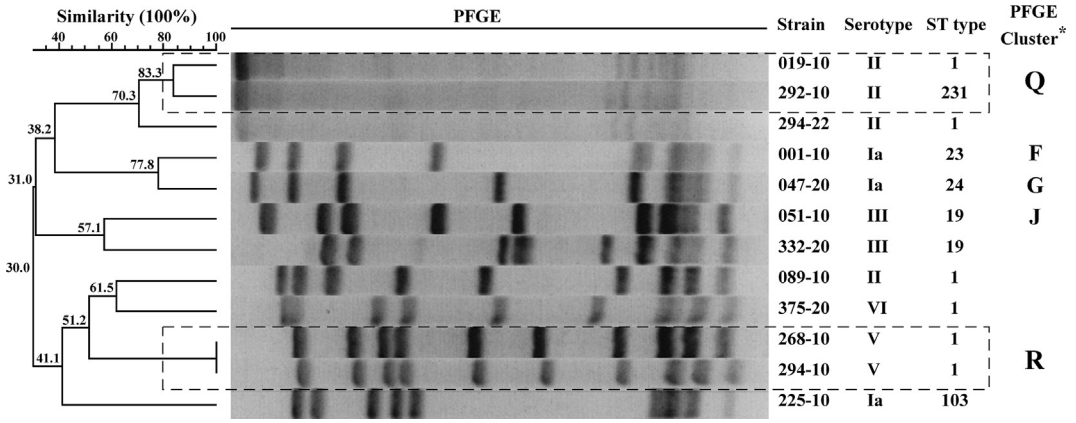

Figure 2 Dendrogram of the PFGE profiles of 12 GBS isolates. Dice similarity coefficient (%) is indicated in scale. Two or more isolates form a cluster with a Dice coefficient of ≧ 80%. *: PFGE cluster is designated as described previously.[10] Clusters F, G, and J were the same as the clusters described earlier,[10] while in this study we identified two new clusters Q and R.

88% of invasive GBS isolates from neonates and adults, respectively.[25,26] Early- and late-onset disease is particularly attributed to serotypes III (mainly represented by ST19 in Asia and ST17 in Europe) and Ia (mainly represented by ST23 and ST24).[12,27] We found that serotype II isolates (represented by ST1, belonging to CC1) colonized neonates readily and in some cases they persisted in neonates up to 6 months; however, serotype II GBS causing late-onset and very-late-onset disease rarely occurred.[27,28] Another common serotype colonizing neonates was serotype III, as described above, and was the most common serotype to cause late-onset disease in neonates. This implied that different GBS serotypes, differing in capsular polysaccharide composition, might have different virulence in humans.

In our study, the follow-up monitoring of GBS colonization for 9 months demonstrated that serotypes II (represented by ST1 and ST231) and III (represented by ST19) could colonize neonates for longer periods than other serotypes (Ia, V and VI), indicating that serotypes II and III might not only be able to cause early-onset disease but also late-onset and very late-onset diseases. In contrast, serotypes Ia, V, and VI may mainly cause early-onset disease because they are more easily cleared over time by the host than serotypes that cause late-onset disease. The long period of colonization by serotype III, with other serotypes having shorter periods of colonization, is consistent with previous findings by Martins et al. Moreover, serotype III CC17 (also ST17) isolates are predominant in Portugal while serotype III CC19 isolates were predominant in this study and elsewhere in Asia.[12,27] The clonal complexes of the main GBS isolates in this study were CC1, CC19, and CC23, which is in accordance with the results of eBURST analysis in Japan (main clonal complexes CC1, CC19, and CC23),[28] China (CC19, CC23, and CC10),[12] the US (CC19, CC23, and CC1),[29] and Europe (CC17, CC23, and CC19).[27]

GBS-positive pregnant women and neonates are treated using appropriate antimicrobial agents such as penicillin and clindamycin. GBS isolates are mostly sensitive to penicillin, although reduced susceptibility to penicillin has been reported since 2008.[30,31] Clindamycin or erythromycin is recommended for GBS intrapartum prophylaxis in penicillin-allergic women with a high risk of anaphylaxis or when therapeutic failure is suspected.[8] However, resistance rates to clindamycin and erythromycin vary dramatically between genetic types and geographic regions in Europe, Asia, North America, and South America.[32–34] For example, the rate of resistance of GBS isolates is 15.3–17.0% (mainly ST17) in Portugal,[27] 50.6–69.0% (mainly ST19, ST23, ST12, and ST1) in China,[12] and 41.7% (mainly ST1, ST19, and ST103) in Taiwan in this study. Our GBS strains isolated from healthy neonates had similar features to those isolated from clinical GBS strains in China, indicating that our GBS isolates could occasionally be potential pathogens in neonates.

The switch in GBS serotype (from serotype V to serotype II) found in a single neonate in this study demonstrated that two separate GBS isolates can independently colonize different sites at different times. In addition, our isolation, from healthy neonates, of three GBS strains with the same PFGE patterns, serotypes and sequence types as those for some previously reported clinical isolates demonstrated that the colonizing GBS strain in a healthy neonate could potentially cause GBS infection. This observation suggested that GBS infection in neonates could be caused by vertical transmission from mother to newborn during labor or by horizontal transmission postnatally from an unknown environmental reservoir. As reported by Zimmermann et al. (2017), breast feeding is a predisposing factor for late-onset disease with a higher associated recurrence rate of GBS infection, compared to newborns fed milk from other potential sources.[35] While IAP can be used to prevent early-onset sepsis,[36] late-onset GBS disease remains difficult to control. For a better understanding of neonatal GBS infection, further studies to decipher the mechanism of GBS transfer between mother and neonate, at different times and sites, are still needed.

5. Conclusions

The GBS carriage rate of healthy neonates born to GBS-colonized (23.7%) or non-colonized (76.3%) women still showed 2.2%, although IAP that was reported to be

effective in lowering down early-onset GBS disease was used for the colonized women in this study. The GBS carriage period among the healthy GBS-positive neonates only lasted less than one month in most infants. The information obtained is important for devising better prevention and treatment strategies against GBS infections.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.07.015.