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Major Article

Neonatal colonization of group B *Streptococcus* in China: Prevalence, antimicrobial resistance, serotypes, and molecular characterization

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Background: Group B *Streptococcus* (GBS) remains a leading cause of neonatal mortality and morbidity. This study aimed to determine the prevalence, antimicrobial susceptibility, serotypes, and molecular characterization of GBS colonized in neonates.

Methods: A cross-sectional study was conducted using a multistage sampling method. Swabs for GBS identification were taken from infants' ear, oral cavity, and umbilicus immediately after birth. All GBS isolates were tested for antimicrobial susceptibility, resistance genes, serotyping, multilocus sequence typing, and virulence genes.

Results: Of the 1,814 neonates, 1.3% tested positive for GBS, with 66.7% tested as multidrug resistant. All GBS isolates were susceptible to penicillin, but rates of resistance to tetracycline and erythromycin were high (70.8%), with the predominant resistance genes being *tetM* and *ermB*. The predominant serotype was III, followed by Ia and Ib, and the most common genotypes were sequence type (ST) 19, ST10, and ST485. Notably, we found that ST19 and ST17 isolates were associated with serotype III, resistant to tetracycline, erythromycin, and clindamycin, and carrying *ermB*, *tetM*, and *rib*; ST10 and ST12 isolates were associated with serotype Ib, resistant to erythromycin and clindamycin, and carrying *ermB* and *alphaC*; and ST485 isolates were associated with serotype Ia and carrying *mefA*/*E*, *tetM*, and *epsilon*.

Conclusions: These findings indicate a high prevalence of multidrug-resistant GBS and specific phenotype-genotype combinations for GBS clones.

Group B *Streptococcus* (GBS) (*Streptococcus agalactiae*) is a significant human pathogen that causes neonatal pneumonia, sepsis, and meningitis, which may present in neonates and infants as early onset disease or late-onset disease.[1,2] Despite a recent decline in incidence, GBS remains a leading cause of neonatal mortality and morbidity in many parts of the world.[3,4] GBS colonizes the gastrointestinal and genital tracts of approximately 25% of healthy pregnant women.[1,2] Neonatal colonization or disease results from transmission from a GBS-colonized mother to her newborn via the ascending route during labor and delivery.[1]

It is well known that GBS colonization is associated with increased risks for nosocomial and community-associated infections with this organism. A number of epidemiologic studies have reported the prevalence of GBS colonization in neonates, suggesting considerable geographic differences.[5-7] However, limited data on neonatal GBS colonization are available for Asian countries. In addition, many challenges exist to obtain the prevalence rate, antimicrobial susceptibility, and molecular characteristics of neonatal GBS isolates, including the logistical challenge of obtaining specimens from newborns soon after birth, difficulties in collecting an optimal volume of blood for culturing, and lack of appropriate laboratory facilities for GBS diagnosis and genotyping, especially for low- and middle-income countries.

The primary aim of this study was to assess the prevalence, antimicrobial susceptibility, serotypes, and molecular characterization

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of GBS isolated from neonates immediately after birth. The secondary aim was to distinguish GBS clones based on phenotypic and genotypic characteristics.

METHODS

Study population and samples collection

This cross-sectional study was conducted in Shenzhen city, China, from July-November 2015. The target population was newborns, and a multistage stratified sampling process was used to obtain a representative sample. First, all the districts were divided into 2 categories (urban and rural areas). One district was randomly sampled from each category. Second, one hospital was randomly drawn from each sampled district. Third, newborns were recruited from the sampled hospitals. The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and it was performed in accordance with the approved guidelines. After obtaining informed consent from their mothers, neonatal specimens for GBS were sampled from the ear canal, oral cavity, and umbilicus by trained nurses immediately after birth.

GBS identification

Swabs were inoculated into selective media (Trans-Vag broth with 8 μg/mL gentamicin and 15 μg/mL nalidixic acid) at 4°C during transportation and inoculated at 37°C with the addition of 5% CO_2 for 24 hours. Then a loopful of the broth was subcultured onto 5% sheep blood agar and incubated at 37°C in 5% CO_2 for 24 hours. Isolates were confirmed to be GBS by a combination of gram staining, morphology, hemolysis pattern, catalase test, and Christie-Atkins-Munch-Peterson (CAMP) test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute.[8] The following antimicrobial disks were used: penicillin, ceftriaxone, levofloxacin, clindamycin, erythromycin, chloramphenicol, tetracycline, linezolid, and vancomycin. GBS isolates were classified as multidrug resistant if they were nonsusceptible (including both intermediate and resistant isolates) to ≥3 classes of antimicrobials.[9] In addition, polymerase chain reaction assays were used to detect tetracycline-resistant genes (*tetM*, *tetO*, *tetL*, and *tetK*), macrolide-resistant genes (*ermA*, *ermB*, *ermTR*, and *mefA/E*), and lincosamide-resistant gene (*linB*).[10,11]

Molecular characterization

Multilocus sequence typing (MLST) was used to type GBS that involves sequencing the 7 housekeeping genes.[12] Then, the sequence types (STs) and allelic profiles were confirmed by querying the MLST database (http://eburst.mlst.net). STs were clustered into a clonal complex (CC) by using the eBURST software program (Department of Infectious Disease Epidemiology, Imperial College London, London, UK; http://eburst.mlst.net), and the term singleton ST refers to an ST that did not cluster into a CC. The GBS serotypes (Ia, Ib, and II-IX) based on *cps* were distinguished using polymerase chain reaction methods.[13] The presence of *alp* genes encoding alpha-like protein (*epsilon*, *alp2/3*, *alp4*, *alphaC*, and *rib*), adhesion-related gene (*scpB*), and invasion-related gene (*hylB*) were determined as previously described.[14-16]

Data analysis

All data were entered in duplicate into EpiData version 3.1 database (The EpiData Association, Odense, Denmark). The relations between STs and serotypes of GBS isolates were illustrated by the minimum spanning tree (PHYLOVIZ software version 2.0; PHYLOViZ team, Lisboa, Portugal). Categorical variables were compared by the Fisher exact test. These analyses were performed using STATA version 13.0 (StataCorp, College Station, TX). A 2-sided *P* value of ≤.05 was considered to be statistically significant.

RESULTS

Participant characteristics

Between July and November 2015, a total of 2,135 newborns were eligible for enrollment. Two caregivers did not provide consent, and neonates of the remaining 2,133 consenting caregivers were included in this study. Of these 2,133 neonates, 1,814 (85.0%) neonates had both obstetric data and bacteriologic cultures. The overall prevalence of GBS colonization in neonates was 1.3% (24/1,814). There were no significant differences in basic and clinical characteristics (including sex, weight, malformation, fever, dyspnea, sepsis, and pneumonia) between GBS-negative and GBS-positive neonates (Table 1).

Table 1
Characteristics of GBS-negative and GBS-positive neonates

Characteristics		Total (N = 1,814)	GBS-negative (n = 1,790)	GBS-positive (n = 24)	*P* value
Sex	Male	981	965 (98.4)	16 (1.6)	.213
	Female	833	825 (99.0)	8 (1.0)	
Weight (g)	~1,000	2	2 (100.0)	0 (0.0)	.978
	~1,500	51	50 (98.0)	1 (2.0)	
	~2,500	1,679	1,657 (98.7)	22 (1.3)	
	≥4,000	82	81 (98.8)	1 (1.2)	
Clinical diagnoses					
Malformation	Yes	10	10 (100.0)	0 (0.0)	>.999*
	No	1,804	1,780 (98.7)	24 (1.3)	
Fever	Yes	4	4 (100.0)	0 (0.0)	>.999*
	No	1,810	1,786 (98.7)	24 (1.3)	
Dyspnea	Yes	19	19 (100.0)	0 (0.0)	>.999*
	No	1,795	1,771 (98.7)	24 (1.3)	
Sepsis	Yes	140	138 (98.6)	2 (1.4)	.909
	No	1,674	1,652 (98.7)	22 (1.3)	
Pneumonia	Yes	1	1 (100.0)	0 (0.0)	>.999*
	No	1,813	1,789 (98.7)	24 (1.3)	

NOTE. Values are n (%) or as otherwise indicated.
GBS, group B *Streptococcus*.
**P* values were calculated with Fisher exact test.