

Characterization of Group B Streptococcus Recovered from Pregnant Women and Newborns Attending in a Hospital in Beijing, China

Xiaoou Li¹, Wei Gao², Zhonglan Jia³, Kaihu Yao², Jinyan Yang¹, Jingjing Tong⁴, Yajuan Wang⁵

¹Department of Neonatology, Haidian District Maternal and Child Health Care Hospital, Beijing, 100080, People's Republic of China; ²Key Laboratory of Major Diseases in Children, Ministry of Education, National Key Discipline of Pediatrics (Capital Medical University), Laboratory of Dermatology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, 100045, People's Republic of China; ³Department of Clinical Laboratory, Haidian District Maternal and Child Health Care Hospital, Beijing, 100080, People's Republic of China; ⁴Department of Neonatology, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, 100026, People's Republic of China; ⁵Department of Neonatology, Children's Hospital, Capital Institute of Pediatrics, Beijing, 100020, People's Republic of China

Correspondence: Yajuan Wang, Department of Neonatology, Children's Hospital, Capital Institute of Pediatrics, No. 2 Yabao Road, Chaoyang District, Beijing, 100020, People's Republic of China, Tel +86-10-85695631, Email cxswyj@vip.sina.com

Purpose: We investigate the drug resistance, serotype and multilocus sequence typing (MLST) of Group B streptococcus (GBS) strains obtained from pregnant women and neonates in a hospital in Beijing.

Patients and Methods: In this cross-sectional study, 1470 eligible pregnant women at a gestational age of 35–37 weeks presented to our department between May 2015 and May 2016 were included. Vaginal and rectal samples from pregnant women together with sampling from neonatal samples were collected to screen GBS. GBS strains were subject to drug resistance and serotype analysis and MLST.

Results: GBS strains were isolated from 111 pregnant women (7.6%) and 6 neonates (0.99%) from 606 matched neonates. 102 strains from pregnant women and 3 strains from neonates were included in the drug sensitivity test, serotyping and MLST typing. All these strains were susceptible to ampicillin, penicillin, ceftriaxone, vancomycin, linezolid, and meropenem. Sixty strains (58.8%) showed multi-drug resistance. Serious cross-resistance was seen between erythromycin and clindamycin. There were eight serotypes, and 37 strains (36.3%) showed a serotype of type III serving as the major type. All 102 GBS strains isolated from pregnant samples could be divided into 18 STs types. They belonged to five clonal complexes and five single clones, with the predominant type of ST19/III, ST10/Ib, and ST23/Ia, with CC19 as the most common type. Three GBS strains isolated from neonates covered two serotypes (ie type III and Ia) that were consistent with those of the mothers.

Conclusion: Serotype III was the predominant serotype of GBS in this study. The predominant MLST type was ST19, ST10, and ST23, with ST19/III, ST10/Ib, and ST23/Ia serving as the most prevalent and CC19 as the most common clonal complex. GBS strains from neonates were consistent in the clonal complex, serotype, and MLST with these isolated from the mothers.

Keywords: Group B streptococcus, multilocus sequence typing, drug resistance, molecular epidemiology

Introduction

Group B streptococcus (GBS), a gram-positive bacterium commonly colonized in the gastrointestinal and genitourinary tracts, is the leading cause of serious neonatal infection.¹ According to the CDC guidelines, GBS screening is recommended at a gestational age of 35–37 weeks, and most GBS infection can be prevented using universal screening followed by intrapartum antimicrobial prophylaxis (IAP).² The utilization of IAP reduces the incidence of early onset GBS disease (GBS-EOD) significantly, but antibiotics may also trigger infection of drug-resistant strains. Penicillin has been preferred for the prevention and treatment of GBS infection as GBS strains are susceptible to penicillin.³ However, there are few studies reporting decreased susceptibility to penicillin.^{4,5} Indeed, the application of antibiotics triggers significant decline in the postnatal infection and neonatal diseases, together with the incidence of severe conditions and even mortality, but it may increase the

risk of drug resistance and dissemination. Meanwhile, a high proportion of GBS is resistant to erythromycin, clindamycin, tetracycline, and quinolones, which raises a challenge for the management of GBS infection.⁶

In mainland China, the colonization rate of GBS varies in different geographic locations, and there is no exact consensus on the GBS screening in China. In a recent study, the GBS colonization rate in China is in a range of 3.7%–14.5%, and the incidence of invasive GBS disease in infants was 0.55–1.79 per 1000 live births.⁷ In a multicenter prospective study, GBS screening is recommended as a routine neonatal screening in southern China with a high colonization rate, while in northern China showing a low GBS colonization rate, a high-risk factor evaluation plan should be adopted.⁸ Recently, GBS screening and IAP have been gradually carried out in Chinese hospitals, to obtain the characteristics of GBS colonization along with establishing prevention strategies for neonatal GBS-EOD.

Currently, the molecular epidemiology of GBS has been highly reliant on multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), as well as whole-genome sequencing (WGS). To date, at least 10 GBS serotypes (ie Ia, Ib, and II–IX) have been identified from GBS⁹. GBS vaccine for females of childbearing age is a new option for the prevention and treatment of GBS infection. Capsular polysaccharide (CPS), as a major virulence factor for GBS, plays an important role in the research and development of GBS vaccine. As serotyping of GBS was different according to geographical location, season, race, and pregnancy status of the women,⁹ it is necessary to investigate the serotype before the preparation of capsular polysaccharide vaccine.

In this study, GBS screening was performed on pregnant women presented to a local hospital in Beijing city. This study was designed to investigate the serotype and drug resistance of GBS strains obtained from pregnant women in Beijing city. In addition, based on serotype analysis and MLST, we conducted a molecular epidemiology study for the GBS strains in Beijing city, and analyzed the homology between GBS colonized in neonates and mothers.

Materials and Methods

Subjects

In this cross-sectional analysis, we included 1500 pregnant women at a gestational age of 35–37 weeks presented to the Outpatient Department, Haidian Maternal, and Child Health-care Hospital between May 2015 and May 2016. The inclusion criteria were as follows: i) pregnant women underwent prenatal screening at a gestational age of 35–37 weeks in the obstetrics clinic; ii) with no history of vaginal medication and lavage and no history of antibiotics and hormone medication in the past 1 week. Those not willing to receive sample collection using vaginal swab and rectal swab were excluded from this study. Vaginal and rectal samples were obtained from each subject using vaginal swab and rectal swab (Copan, USA), according to the previous description.³ For neonates, a surface swab sample was collected from the navel, nostril, or cochlea of the newborns. Vaginal, rectal, and surface swab samples were stored in three different transport media at –20°C. Afterwards, the samples were subject to GBS antimicrobial susceptibility analysis, serotyping, and MLST analysis. Samples from 1470 pregnant women were finally included in this study after excluding 30 samples not underwent delivery in our hospital.

GBS Strains Screening

Selective LIM Broth enrichment medium was used for the isolation of GBS strains, according to the conventional description.¹⁰ Strains collected from vaginal and/or rectal samples of pregnant women that were identified as GBS positive using streaking method were subject to serotype analysis. Strains isolated merely from vaginal or rectal samples were subject to subsequent analysis directly. For strains isolated from both vaginal and rectal samples with consistent serotypes, subsequent analysis was performed using GBS strains obtained from rectal samples. Among the neonates born by these pregnant women, 606 neonatal samples from 606 neonates were collected in total from the peripheral umbilical cord, nostril, or cochlea, and then the strains were subject to serotype and molecular typing analysis (Figure 1).

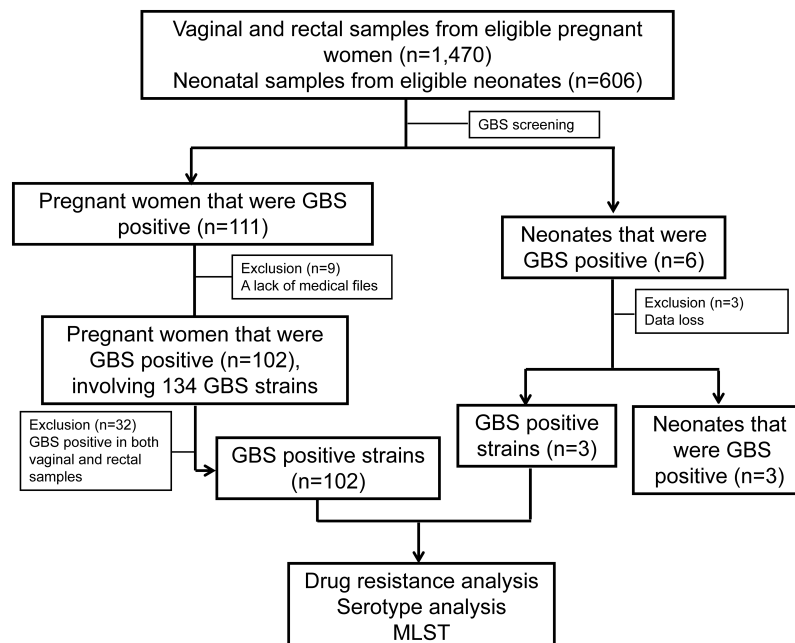


Figure 1 Flowchart of GBS strains screening from the subjects.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility analysis was performed for the GBS strains from pregnant samples and neonatal samples, respectively. The test was performed using dilution method based on the previous description.¹¹ Briefly, 10 antibiotics, including ampicillin, penicillin, erythromycin, clindamycin, vancomycin, ceftriaxone, tetracycline, levofloxacin, linezolid, and meropenem, were utilized for the antimicrobial susceptibility test. Finally, the antimicrobial susceptibility analysis was performed using the Microbial ID/AST System DL-96II (Zhuhai, China). The test was performed in accordance with Clinical and Laboratory Standards Institute guidelines. *Staphylococcus aureus* ATCC 25923 and *Streptococcus agalactiae* ATCC12386 served as the quality control strains. Strains with simultaneous resistance to at least three classes of antimicrobial agents were defined as multidrug-resistant GBS (MDRGBS).

Serotype Analysis

The serotype analysis was performed using the Streptococcal grouping kit (OXOID, UK) according to the previous description.¹² Serums (ie Ia, Ib, and II–IX) were purchased from Statens Serum Institut (Copenhagen, Denmark). The strains were defined as non-typeable (NT) in cases of no aggregation between the strain solution and the serum samples.

MLST

DNA was extracted using a commercial kit provided by SBS Biotech (Beijing, China). PCR was utilized to amplify the housekeeping genes including *adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkf* of GBS, using specific primers downloaded from MLST database (<https://pubmlst.org/organisms/streptococcus-agalactiae/>). After purification, sequencing results were submitted to the MLST genotype database (<http://pubmlst.org/sagalactiae/>). The eBURST v3 software (<http://eburst.mlst.net>) was utilized to analyze the relationship between the strains. The clonal complex was defined unless there were six consistent alleles among the seven counterparts.

Analysis of eBURST

eBURST was performed for a more comprehensive analysis of the possible patterns of evolutionary descent. The analysis was performed according to the previous description.¹³ Each genotype cluster was identified using the goeBURST algorithm version 1.2.1 (<http://www.phyloviz.net/goeburst/>).

Results

Strain Characteristics

Among the 1470 pregnant women, GBS was detected in the vaginal or rectal samples from 111 pregnant women yielding a colonization rate of 7.6%. After excluding nine pregnant women with a lack of medical files, 102 were proved to be GBS positive. In total, 134 GBS strains were isolated from 102 pregnant women. The 102 GBS strains from 102 pregnant women were subject to antimicrobial susceptibility and MLST type. In addition, six neonates were proved to be GBS positive among the 606 samples, yielding a colonization rate of 0.99%. Finally, three strains from three neonates who underwent all tests were obtained.

Serotype Analysis

Among the 102 GBS strains from the pregnant women, 37 strains (36.3%) showed a serotype of type III serving as the major type, followed by type Ib (21 strains, 20.6%), type Ia (17 strains, 16.7%), type V (13 strains, 12.7%), type VI (3 strains, 2.9%), type VIII (3 strains, 2.9%), type II (2 strains, 2.0%), and type IX (2 strains, 2.0%). In total, eight serotypes (ie Ia, Ib, II, III, V, VI, VIII, and IX) were identified. The other four strains (3.9%) were defined as NT as they showed no aggregation to the ten serotypes. Three strains isolated from the neonates covered two serotypes including type III (n=2) and Ia (n=1). The strains showed consistent serotypes with the GBS serotypes collected by vaginal or rectal swabs. Three GBS strains isolated from neonates covered two serotypes (ie type III and Ia) that were consistent with those of the mothers (Figure 2).

MLST Analysis

For the MLST analysis, all the 102 GBS strains isolated from the pregnant samples could be divided into 18 STs types. Among these strains, 89 (87.3%) were classified into 8 ST types involving at least 3 strains in each type. The major ST type was ST19 (30 strains, 29.4%), followed by ST10 (15 strains, 14.7%), ST1 (11 strains, 10.8%), ST23 (11 strains, 10.8%), ST12 (7 strains, 6.9%), ST17 (7 strains, 6.9%), ST485 (5 strains, 4.9%), and ST4 (3 strains, 2.9%) (Table 1).

Analysis of eBURST

The eBURST software was utilized to analyze the relationship of the strains. As shown in Figure 3, there were five clonal complexes (CC19, CC10, CC17, CC485, and CC890) and five single clones (ie ST1, ST23, ST4, ST651, and ST929) among the 102 strains. The five CC were designated as CC19 involving 31 strains (30.4%), CC10 involving 26 strains (25.5%), CC17 involving 8 strains (7.8%), CC485 involving 7 strains (6.9%), as well as CC890 involving 3 strains (2.9%). The five single clones were designated as ST1 involving 11 strains (10.8%), ST23 involving 11 strains (10.8%), ST4 involving 3 strains (2.9%), ST651 involving 1 strain (1.0%) and ST929 involving 1 strain (1.0%) (Table 1) (Supplementary Table 1).

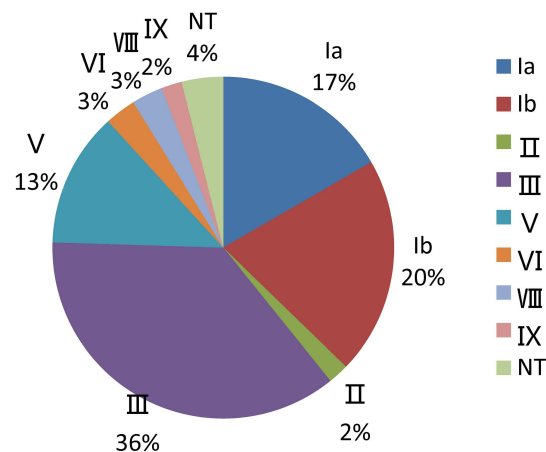


Figure 2 Serotyping results for the 102 GBS strains from the pregnant women.

Table 1 Association Between Serotype and GBS Clone in the 102 GBS Strains

GBS clone	Number of strains	Serotype								
		Ia	Ib	II	III	V	VI	VIII II II	IX	NT
CC19	31, 30.4%	0	0	0	25	3	0	0	1	2
ST19	30, 29.4%	0	0	0	24	3	0	0	1	2
ST193	1, 1.0%	0	0	0	1	0	0	0	0	0
CC10	26, 25.5%	1	21	1	0	0	0	1	0	2
ST10	15, 14.7%	0	12	0	0	0	0	1	0	2
ST12	7, 6.9%	1	6	0	0	0	0	0	0	0
ST8	2, 2.0%	0	2	0	0	0	0	0	0	0
ST358	1, 1.0%	0	1	0	0	0	0	0	0	0
ST509	1, 1.0%	0	0	1	0	0	0	0	0	0
CC17	8, 7.8%	0	0	0	8	0	0	0	0	0
ST17	7, 6.9%	0	0	0	7	0	0	0	0	0
ST188	1, 1.0%	0	0	0	1	0	0	0	0	0
CC485	7, 6.9%	2	0	0	3	0	0	2	0	0
ST485	5, 4.9%	2	0	0	1	0	0	2	0	0
ST862	2, 2.0%	0	0	0	2	0	0	0	0	0
CC890	3, 2.9%	0	0	0	0	3	0	0	0	0
ST24	2, 2.0%	0	0	0	0	2	0	0	0	0
ST890	1, 1.0%	0	0	0	0	1	0	0	0	0
ST1	11, 10.8%	2	0	1	0	5	3	0	0	0
ST23	11, 10.8%	10	0	0	0	0	0	0	1	0
ST4	3, 2.9%	2	0	0	0	1	0	0	0	0
ST651	1, 1.0%	0	0	0	1	0	0	0	0	0
ST929	1, 1.0%	0	0	0	0	1	0	0	0	0
Total	102, 100%	17	21	2	37	13	3	3	2	4

Correlation Between GBS Clone, MLST, and Serotypes

In this section, we analyzed the correlation among GBS clone, MLST, and serotypes. Seventeen strains showed a serotype of Ia, of which the majority were classified into ST23 (58.8%), ST1 (11.8%), ST4 (11.8%), CC10 (5.9%), 1

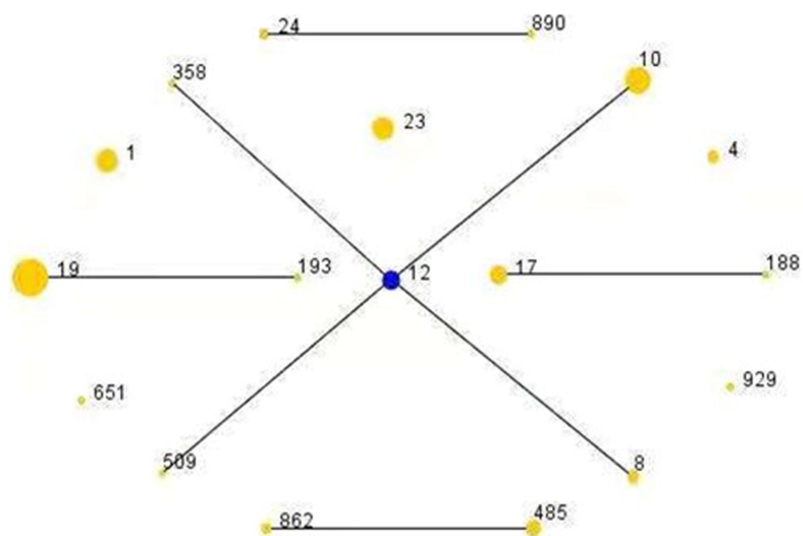


Figure 3 The eBURST analysis results for the 102 GBS strains.

strain with ST12), and CC485 (11.8%, 2 strains with ST485), respectively. Twenty-one strains presented a serotype of Ib, which were all categorized into CC10 including ST10 (12 strains), ST12 (6 strains), ST8 (2 strains), and ST358 (1 strain). Two strains showed a serotype of II including one strain with a serotype of ST1 and 1 strain of CC10 (1 strain with ST509).

The strains of type III were categorized into CC19 (67.6%, 24 strains with ST19 and 1 strain with ST193), CC17 (21.6%, 7 strains with ST17 and 1 strain with ST188), CC485 (8.1%, 1 strain with ST485 and 2 strains with ST862), and ST651 (2.7%, 1 strain). The strains of type V were categorized into ST1 (5 strains, 38.5%), CC19 (3 strains with ST19, 23.1%), CC890 (23.1%, 2 strains with ST24 and 1 strain with ST890), ST4 (1 strain, 7.7%), and ST929 (1 strain, 7.7%). Three strains showed a serotype of VI, which were all categorized into ST1 type. The strains of VIII type were categorized into CC485 (66.7%, 2 strains with ST485) and CC10 (33.3%, 1 strain with ST10). Two strains of type IX were classified into CC19 (50.0%, 1 strain with ST19) and ST23 (50.0%). The NT strains were categorized into CC19 (50.0%, 2 strains with ST19) and CC10 (50.0%, 2 strains with ST10), respectively (Table 1). The three GBS strains from the neonatal samples were categorized into CC17 (serotype III/ST17), CC19 (serotype III/ST19), CC23 (serotype Ia/ST23), which showed consistent clonal complex, MLST, and serotype, respectively.

GBS Antimicrobial Susceptibility Analysis

Then, GBS drug sensitivity analysis was given to the strains of five clonal complexes (CC19, CC10, CC17, CC485, and CC890) and five single clones (ie ST1, ST23, ST4, ST651, and ST929), respectively. All 102 GBS strains from pregnant women were susceptible to ampicillin, penicillin, ceftriaxone, vancomycin, linezolid, and meropenem. Seventy-three strains were designed as “intermediate” (1 strain) and “resistant” (72 strains) in the antimicrobial susceptibility analysis to erythromycin. Fifty-three strains were intermediate (3 strains) and resistant (50 strains) to clindamycin. Eighty strains (78.4%) were resistant to tetracycline, and 46 strains (45.1%) were resistant to levofloxacin (Table 2).

Antimicrobial Susceptibility of the GBS Strains to the Antibiotics

Among the 102 strains, 96 (94.1%) were resistant to at least one type of antibiotics utilized in this study (Table 3). In total, 60 (58.8%) were resistant to at least three classes of antibiotics, including 13 with simultaneous resistance to erythromycin, clindamycin, tetracycline, and levofloxacin, 23 with simultaneous resistance to erythromycin, clindamycin, and tetracycline, 14 with simultaneous resistance to erythromycin, clindamycin, and levofloxacin, 9 with simultaneous resistance to erythromycin, tetracycline, and levofloxacin, as well as 1 with simultaneous resistance to clindamycin, tetracycline, and levofloxacin, respectively. Twenty-three strains were resistant to two antibiotics, including 12 with simultaneous resistance to erythromycin and tetracycline, 9 with simultaneous resistance to tetracycline and levofloxacin, and 2 with simultaneous resistance to erythromycin and clindamycin. Thirteen strains were resistant to one type of antibiotics (tetracycline). Among the 73 strains that were not susceptible to erythromycin, 52 were resistant to clindamycin, which was significantly higher than that of the resistance rate among the erythromycin-susceptible strains (71.2% vs 3.4%, $P < 0.05$). Among the 53 strains that were resistant to the clindamycin, 52 were not susceptible to the

Table 2 Drug Sensitivity Test for the 102 GBS Strains

Drug	Susceptible	Intermediate	Resistant	MIC range	MIC50	MIC90
Penicillin	102, 100.0%	0, 0.0%	0, 0.0%	≤0.06–0.12	≤0.06	≤0.06
Ampicillin	102, 100.0%	0, 0.0%	0, 0.0%	≤0.25	≤0.25	≤0.25
Ceftriaxone	102, 100.0%	0, 0.0%	0, 0.0%	≤0.5	≤0.5	≤0.5
Vancomycin	102, 100.0%	0, 0.0%	0, 0.0%	≤1	≤1	≤1
Linezolid	102, 100.0%	0, 0.0%	0, 0.0%	≤2	≤2	≤2
Meropenem	102, 100.0%	0, 0.0%	0, 0.0%	≤0.25	≤0.25	≤0.25
Erythromycin	29, 28.4%	1, 1.0%	72, 70.6%	≤0.25–≥16	4	≥16
Clindamycin	49, 48.0%	3, 2.9%	50, 49.0%	≤0.25–≥8	0.5	≥8
Tetracycline	22, 21.6%	0, 0.0%	80, 78.4%	≤1–≥64	32	≥64
Levofloxacin	56, 54.9%	0, 0.0%	46, 45.1%	2–≥16	2	≥16

Table 3 Drug Resistance for the 102 GBS Strains

Condition	Number of Strains
Erythromycin, clindamycin, tetracycline, levofloxacin	13
Erythromycin, clindamycin, tetracycline	23
Erythromycin, clindamycin, levofloxacin	14
Erythromycin, tetracycline, levofloxacin	9
Clindamycin, tetracycline, levofloxacin	1
Erythromycin, tetracycline	12
Tetracycline, levofloxacin	9
Erythromycin, clindamycin	2
Tetracycline	13
No tolerance to these agents	6

erythromycin, which was significantly higher than the resistance rate among the clindamycin-susceptible strains (98.1% vs 42.9%, $P < 0.05$). This indicated the presence of severe cross resistance.

Drug Resistance Analysis of GBS Strains of Different CC and MLST to Antibiotics

The strains with a serotype of CC17, CC890, ST23, ST4, ST651, and ST929 were all susceptible to levofloxacin, while strains of CC19, CC10, CC485, and ST1 showed a resistance rate of 96.8%, 53.8%, 14.3%, and 9.1% to the levofloxacin, respectively

Table 4 Drug Resistance of the 102 Strains Categorized Based on the GBS Clone

GBS Clone	Antibiotic Resistance			
	Erythromycin	Clindamycin	Tetracycline	Levofloxacin
CC19, n=31	22, 71.0%	14, 45.2%	31, 100%	30, 96.8%
CC10, n=26	25, 95.2%	24, 92.3%	10, 38.5%	14, 53.8%
CC17, n=8	7, 87.5%	4, 50.0%	8, 100%	0, 0%
CC485, n=7	2, 28.6%	2, 28.6%	5, 71.4%	1, 14.3%
CC890, n=3	1, 33.3%	1, 33.3%	3, 100%	0, 0%
ST1, n=11	6, 54.5%	4, 36.4%	9, 81.8%	1, 9.1%
ST23, n=11	7, 63.6%	2, 18.2%	10, 90.9%	0, 0%
ST4, n=3	2, 66.7%	2, 66.7%	3, 100%	0, 0%
ST651, n=1	0, 0%	0, 0%	0, 0%	0, 0%
ST929, n=1	1, 100%	0, 0%	1, 100%	0, 0%
Total, n=102	73	53	80	46

Table 5 Levofloxacin Resistance of GBS Strains with a Serotype of III

GBS Clone Type	Strains with Serotype III	Resistance Rate Among the Serotype III Strains
CC19, n=31	25	24, 96.0%
ST19, n=30	24	23, 95.8%
ST193, n=1	1	1, 100%
CC17, n=8	8	0
ST17, n=7	7	0
ST188, n=1	1	0
CC485, n=7	3	1, 33.3%
ST485, n=5	1	1, 100%
ST862, n=2	2	0
ST651, n=1	1	0
Total n=47	37	25, 67.6%

(Table 4). As III/ST19 was the major type for CC19 strains, we then analyzed the drug resistance of III/ST19 strains, which yielded a resistance rate of 95.8% to the levofloxacin. Among the levofloxacin-resistant strains, the majority of strains (65.2%) were of CC19 type (Table 4). The III/ST17 strains were all susceptible to levofloxacin (Table 5).

Discussion

In this study, we focused on the serotype, MLST, and drug resistance of the GBS strains obtained from pregnant women in Beijing city. Then, based on serotype analysis and MLST, we conducted a molecular epidemiology study for the GBS strains in Beijing city. Our data showed that serotype III was the predominant serotype (38.3%) of GBS colonized in pregnant women in Beijing in northern China, followed by Ib (20.2%), Ia (18.1%), V (10.6%), and II (1.1%). Similarly, Lu et al reported that the GBS serotype in 201 pregnant women in Beijing was predominant by III (41.8%), followed by Ia (21.4%), Ib (11.9%), V (14.9%), and II (7.0%).¹⁴

In a previous study, Yan et al investigated the serotype distribution and resistance genes profile in GBS isolated from pregnant women in Shanghai in East China.¹⁵ Seven serotypes (ie Ia, Ib, II, III, V, VI, and VIII) were identified, and III, V, and Ia serotypes were the predominant serotypes. In addition, in a study performed in Xiamen in southern China, Lin et al¹⁶ investigated the serotype features of GBS vaginal colonization in late pregnancies and their relationship with early-onset neonatal GBS disease (GBS-EOD). A total of nine serotypes were identified among the 298 strains isolated from the mothers. The most prevalent serotype was III [55.0% (164/298)], followed by Ib [16.4% (49/298)], Ia [11.1% (33/298)], V [9.4% (28/298)], II [5.0% (15/298)], non-typable [NT, 1.0% (3/298)], and VI, VIII, and IX [0.7% (2/298)] in each. These indicated that the major serotypes of GBS in pregnant women in these cities in China were III, Ia, Ib, and V, respectively. In our study, we reported a colonization rate of GBS of 7.6% in pregnant women. We hope to raise the attention of Chinese scholars on GBS screening in prenatal stage.

The major serotypes of GBS in Japan were VI and VIII, while those in Korea were III and Ia. In Southeast Asian countries such as Thailand and Burma, the major serotype was type II.¹⁷ In US and European countries, the predominant serotype was Ia, II, III, and V, while that in Latin America the predominant serotype was III.^{18,19} These indicated that there were geographical or racial differences between the serotypes of GBS. Recently, Russell et al conducted a meta-analysis for the maternal colonization with GBS and serotype distribution worldwide.²⁰ The dataset regarding colonization included 390 articles, 85 countries, and a total of 299,924 pregnant women. The adjusted estimate for maternal GBS colonization worldwide was 18% with regional variation of 11%–35%. Bacterial serotypes I–V account for 98% of identified colonizing GBS isolates worldwide, while the serotype III, associated with invasive disease, accounted for 25% (95% CI, 23–28%). This would be responsible for the pathogenesis of invasive diseases.

According to a previous study,¹⁶ serotype III is the most prevalent GBS serotype in late pregnant women and GBS-EOD neonates, and also the predominant serotype in infants with early-onset meningitis. Serotype Ia could be highly vertically transmitted, while the virulence of serotypes III and Ia strains of GBS is the strongest. In this study, three GBS strains obtained from neonates were classified as type III and Ia, respectively. In the future, more studies involving a large sample size are required to further illustrate the distribution of serotypes of GBS in pregnant women.

Recently molecular epidemiology has been frequently utilized for investigating the mechanism of GBS, which mainly involves genetic tests and structural changes. In this study, a molecular epidemiology study was performed to GBS, using the MLST analysis, in order to comprehensively illustrate the genotype of GBS in pregnant women in Haidian district. The predominant serotypes were ST19, ST10, and ST23 in the GBS strains isolated in Beijing Haidian district, with ST19/III, ST10/Ib, and ST23/Ia serving as the most prevalent and CC19 as the most common clonal complex. ST19/III was the predominant serotype of GBS in Canada and US,^{21,22} while ST23/Ia and ST17/III serotypes were predominant in Italy,²³ Portugal,¹⁸ and Spain.²⁴ It has been well acknowledged that ST17 was closely related to the invasive infection among neonates.²⁵ However, we could not speculate that the low incidence of GBS infection in Beijing was related to the low carrying rate of ST17 type. In this study, the neonates that were positive for GBS in the swab test were all in the colonized group, and showed the same serotype and ST type with that of the maternal samples. GBS colonization in the pregnancy stage was a major risk factor for GBS infection.²⁶ Therefore, it could reflect the necessity of GBS screening for the prevention of neonatal GBS infection. Besides, it could display the carrying status of GBS.

IAP has been considered as a first-line option for the prevention of vertical transmission of GBS and early-stage neonatal GBS infection.³ According to guidelines proposed by CDC, penicillin is preferred in clinical practice, and ampicillin serves as the second-line option. For pregnant women who were allergic to penicillin, administration of cefazolin was recommended in those without angioneurotic edema, distress of respiratory, or urticaria. In the presence of severe allergy, the GBS susceptibility to clindamycin and erythromycin should be determined simultaneously together with the pregestational screening. Clindamycin was recommended in cases of simultaneous susceptibility. If the strains were resistant to clindamycin, vancomycin should be considered. Our data confirmed that penicillin could still serve as the preferred option for preventing GBS infection in Beijing, which showed a MIC range of $\leq 0.06\text{--}0.12\text{mg/L}$, together with MIC50 and MIC90 of less than 0.06mg/L , respectively. Compared with the previous description on the MIC of penicillin, there was no increase in the MIC in Haidian district.²⁷ In the past decade, there have indeed been some reports revealing a decreased susceptibility of GBS to penicillin.^{28–30} Based on the 56 GBS strains collected from Beijing between 2012 and 2013, Tong et al reported a MIC range of $0.008\text{--}0.125\text{ mg/L}$, and the MIC50 and MIC90 were 0.094 mg/L and 0.125 mg/L , respectively.³¹ Our data showed that GBS strains were all susceptible to penicillin, with no tendency to decrease in susceptibility. In the future, more studies are required to focus on the dynamic monitoring of GBS susceptibility to penicillin.

Erythromycin and clindamycin have been recommended for treating infection among pregnant women who are allergic to penicillin. In this study, the resistance rate of GBS to erythromycin and clindamycin was 71.3% and 51.1%, respectively. In USA, Back et al reported the high rates of perinatal Group B Streptococcus clindamycin and erythromycin resistance in an upstate New York hospital, in which the resistance rate of GBS to erythromycin and clindamycin was 50.7% and 38.4%, respectively.³² In a study carried out in Switzerland, Capanna reported that rate of resistance to clindamycin was 28% and to erythromycin was 30%.³³ In Beijing, the resistance of GBS to erythromycin and clindamycin increased from 8.5% and 20.5% in 2001 to 70.8% and 68.8% in 2018, respectively.^{34,35} In line with this, our data showed a significant increase in the erythromycin resistance of GBS strains isolated in Haidian district. Unlike the previous study, our data showed no significant changes in the resistance of GBS to clindamycin. In this study, the resistance rate of GBS to levofloxacin was 48.9%, which showed slight increase compared with the previous data in 2015 (37.7%), which was significantly higher than that of Taiwan and USA in a range of 0.7% to 1.3%.³⁶ Quinolones antibiotics have been commonly utilized in clinical practice and agriculture for bacterial control. Their extensive application contributes to the resistance of GBS to levofloxacin to some extent.

For the limitation of this study, this is a single-centered study with a small sample size. In the future, studies of large sample sizes are required. Due to the low percentage of GBS recovery, we could not exclude the possibilities of operation difference that may induce non-enrichment of swabs.

In summary, serotype III was the predominant serotype of GBS colonized in pregnant women in Beijing, followed by Ib, Ia, and V. The predominant MLST type was ST19, ST10, and ST23, with CC19 as the most common type. The isolated GBS strains were susceptible to penicillin, cephalosporin, and vancomycin, and were resistant to tetracycline and erythromycin. The GBS strains isolated from neonates were consistent in the clonal complex, serotype, and MLST, with these isolated from pregnant women.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethical Committee of Beijing Children's Hospital, Capital Medical University (2015-62). Informed consent was obtained from all individual participants included in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose.

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