

Serotype Distribution, Antibiotic Resistance Characteristics, and Virulence Factor Analysis of Group B *Streptococcus* Isolates in Guangzhou Based on Whole-Genome Sequencing

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Introduction: *Streptococcus agalactiae* (Group B Streptococcus, GBS) is a Gram-positive opportunistic pathogen and a leading cause of invasive infections in neonates and pregnant women worldwide. Pathogenicity and transmission dynamics are shaped by capsular serotypes, clonal backgrounds, and virulence and resistance determinants, which vary over time and by region. Data from South China remain limited. This study characterized the serotype distribution, sequence types (STs), clonal complexes (CCs), antimicrobial resistance genes, and virulence factors of clinical GBS isolates from Guangzhou.

Methods: GBS-positive clinical isolates collected at Guangdong Provincial People's Hospital (Guangzhou, China) from 2016 to 2022 were subjected to genomic epidemiology analyses. A total of 72 non-duplicate isolates were included. Multilocus sequence typing (MLST) and whole-genome sequencing (WGS) were used to determine capsular serotypes, sequence types (STs) and clonal complexes (CCs), and to identify antimicrobial resistance genes and virulence determinants, including pilus islands.

Results: Among 72 isolates, serotype III predominated (36.1%), followed by serotype V (25.0%). Isolates were assigned to 22 STs within 7 CCs, with ST19 being most common (15.3%). Resistance gene profiling showed *tetM* in 61.1% of isolates and *ermB* in 37.5%. Virulence factor analysis indicated universal presence of *lmb*, *bca*, and *cpsJ*; 97.2% carried the hemolysin gene *hlyB*. Pilus island genes *PI-1* and *PI-2a* were detected in 73.6% of isolates.

Discussion: GBS circulating in Guangzhou shows a serotype distribution dominated by III and V and a diverse clonal structure with ST19 prominence. The high carriage of *tetM* and *ermB* suggests sustained selective pressure for tetracycline and macrolide resistance, while the near-ubiquitous virulence repertoire—including *lmb*, *bca*, *cpsJ*, and *hlyB*—and frequent *PI-1/PI-2a* may support colonization and invasiveness. These findings highlight the need for ongoing, region-specific genomic surveillance to track serotype/lineage shifts and resistance trends and to inform prophylaxis, empiric therapy, and future vaccine coverage in South China.

Keywords: Group B *Streptococcus*, serotype, multilocus sequence typing, resistance gene, virulence factor, whole-genome sequencing

Introduction

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a common Gram-positive bacterium that frequently colonizes the human reproductive and gastrointestinal tracts. The global colonization rate of GBS in pregnant women is approximately 18%, while in Chinese pregnant women, it is 10.5%.^{1,2} During pregnancy and childbirth, GBS can be transmitted vertically to neonates, leading to severe diseases such as sepsis, pneumonia, and meningitis. These infections are major contributors to the increasing morbidity and mortality rates among newborns worldwide.³ GBS colonization is

typically transient and intermittent; however, under specific conditions, it can transform into pathogenic strains, significantly elevating the risk of infection.⁴ In the absence of effective preventive measures, the risk of neonatal GBS infection is markedly increased.^{5,6} Additionally, beyond neonates and pregnant women, elderly individuals and those with compromised immune systems are also susceptible to GBS infections.⁷ Recent studies have indicated that healthy adults may also experience GBS infections.⁸

Antibiotics are currently the primary means for preventing and treating GBS infections, particularly through intrapartum antimicrobial prophylaxis (IAP), which has been proven effective in reducing the incidence of neonatal sepsis, especially early-onset GBS infection (EOD) in GBS-colonized pregnant women.⁹ However, with the widespread use of antibiotics, the issue of antibiotic resistance in GBS has become increasingly prominent. Research has shown a decline in GBS sensitivity to certain antibiotics, especially macrolides represented by the *ermB*, *ermTR*, and *mefA/E* genes.^{10,11} A systematic review and meta-analysis of GBS infection rates and antibiotic resistance in Chinese infants revealed the highest resistance rates for tetracycline (median 98.0%, IQR 80.0–100.0%), followed by clindamycin (median 73.3%, IQR 62.6–78.7%), erythromycin (median 64.4%, IQR 56.6–75.0%), and ciprofloxacin (median 25.0%, IQR 9.1–35.2%).¹² The increasing resistance poses a significant public health challenge, urgently necessitating the development of new prevention and treatment strategies to address resistant GBS strains.

The virulence factors of GBS are complex and diverse, among which capsular polysaccharides (CPS) are critical virulence determinants. Based on CPS variations, GBS isolates are classified into 10 serotypes: Ia, Ib, II–IX.¹³ This classification is of significant importance for the development of CPS-based vaccines. Currently, vaccines targeting different serotypes are under development, including monovalent vaccines, bivalent vaccines (serotypes II and III), trivalent vaccines (serotypes Ia, Ib, and III), and pentavalent vaccines (serotypes Ia, Ib, II, III, and V). With the inclusion of serotype IV, a hexavalent vaccine (I a, I b, II, III, IV, V) is also in the development stage.^{14,15} The hexavalent vaccine offers the broadest coverage; however, the presence of regionally rare serotypes may reduce vaccine efficacy, potentially leading to vaccine-driven serotype replacement. Therefore, continuous monitoring of serotype prevalence trends in different regions is crucial to promptly identify emerging serotypes and adjust vaccine strategies accordingly.¹⁶

In addition to CPS, GBS expresses various other virulence factors such as *lmb*, *cpsM*, and *fbxA*, which play important roles in bacterial adhesion, invasion, and immune evasion. Notably, pili (PI) are surface proteins involved in biofilm formation¹⁷ and facilitate bacterial invasion of host cells. These virulence factors contribute to the multifaceted roles GBS plays in invasive infections, making them potential targets for vaccine development.

Given the substantial heterogeneity of GBS serotypes and virulence factors, as well as their region- and time-dependent epidemiologic variation,^{1,18} systematic, region-specific data curation is warranted. Although global distributions of GBS serotypes have been reported,^{19,20} granular information for southern China—particularly clinical isolates from Guangdong Province—remains limited with respect to serotype composition, sequence types (STs), and antibiotic resistance spectra. In this study, we analyzed multiple GBS-positive clinical isolates collected at Guangdong Provincial People's Hospital, describing serotype distributions, STs, resistance genes, and virulence-factor profiles, and documenting co-occurrence phenomena and potential distributional patterns across these features. These descriptive data provide a regional baseline for the genomic and resistance characteristics of GBS in Guangzhou, offer context for understanding local epidemiologic trends and clinical management, and generate hypotheses to inform subsequent mechanistic and association-focused investigations.

Materials and Methods

Source of Strains

This study collected 72 GBS-positive isolates obtained from the microbiology laboratory of Guangdong Provincial People's Hospital between 2016 and 2022. Duplicate strains from the same patient were excluded.

Microbial Culture and Identification

Microbial culture and identification were conducted according to standard procedures in the hospital's clinical laboratory. Specimens were inoculated onto blood agar plates and incubated at 35°C in a 5% CO₂ environment for 24–48 hours. Selected colonies were purified by sub-culturing under the same conditions for an additional 24–48 hours. Isolates with

β -hemolytic rings were identified using the VITEK MS mass spectrometer, and antimicrobial susceptibility testing was performed with the VITEK[®] 2 Compact system. Once identified as GBS, isolates were cultured on blood agar plates for subsequent whole-genome sequencing.

Whole-Genome Sequencing

Genomic DNA was extracted using the MagPure Bacterial DNA Kit (D6361-02), and libraries were prepared using the Hieff NGS[®] MaxUp II DNA Library Prep Kit for Illumina[®] (YEASEN, 12200ES08). PCR products were purified with magnetic beads (YEASEN, 12601ES56) and checked for quality using 2% agarose gel electrophoresis. Quantification was performed with a Qubit fluorometer, and paired-end sequencing was conducted on the Novaseq 6000 platform (Illumina, San Diego, CA).

Molecular Subtype Analysis

Resistance genes and virulence factors were identified using the CARD and VFDB databases. Serotype determination was performed based on the GBS bioinformatics typing tool from the Centers for Disease Control and Prevention (CDC).

Multilocus Sequence Typing (MLST)

MLST was conducted by sequencing seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkl*).²¹ Based on allele variations, GBS isolates were classified into different STs and further grouped into clonal complexes (CCs). All alleles and STs of the GBS isolates were compared against data in the *S. agalactiae* MLST database (<http://pubmlst.org/sagalactiae>, accessed October 9, 2022) to confirm the corresponding STs and CCs. A minimum spanning tree was constructed using Phyloviz software by integrating CC and serotype data²² and visually refined in Adobe Illustrator 2020.

Results

Source of Specimens

This study collected 72 GBS clinical isolates between 2016 and 2022. Specimen types included genital secretions, midstream clean-catch urine, placenta, conjunctival secretions, wound exudate, venous blood, rectal swabs, synovial fluid, and cerebrospinal fluid. Genital secretions were the most common sample type, accounting for 50% (36/72) of isolates, followed by placenta samples at 18.1% (13/72), and midstream clean-catch urine at 9.7% (7/72). Details of sample types and their distribution are presented in Table 1. These GBS clinical isolates were primarily collected from

Table 1 Distribution of Specimen Types for 72 GBS Isolates

Specimen Type	Number of Isolates (n)	Proportion (%)
Reproductive tract secretions	36	50.0
Midstream clean-catch urine	7	9.7
Placenta	13	18.1
Conjunctival secretions	5	6.9
Wound secretions	1	1.4
Venous blood	1	1.4
Anal swabs	5	6.9
Joint fluid	3	4.2
Cerebrospinal fluid	1	1.4
Total	72	100.0

Abbreviation: n, the number of strains.

Table 2 Clinical Department Distribution of 72 GBS Isolates

Clinical Department	Number of Isolates (n)	Proportion (%)
Obstetrics and Gynecology wards and outpatient clinics	65	90.20
Neurology	2	2.80
Orthopedics and Trauma wards	3	4.20
Rheumatology wards	1	1.40
Concord Ward Area II*	1	1.40
Total	72	100.00

Notes: *A subordinate department under the Concord Advanced Medical Center (Department of General Practice) at Guangdong Provincial People's Hospital.

Abbreviation: n, the number of strains.

obstetrics and gynecology departments and outpatient clinics, accounting for 90.2% (65/72) of all samples. Additionally, three samples were obtained from the departments of orthopedics and trauma, accounting for 4.2% (3/72). Detailed departmental source distributions are provided in [Table 2](#).

Serotype, Sequence Type, and Clonal Complex Analysis

Following an assessment of GBS isolates based on specimen origin, further analysis was conducted on serotype, ST, and clonal complex (CC) to comprehensively reveal their epidemiological patterns and genetic diversity. Results indicate six distinct serotypes among the isolates: Ia, Ib, II, III, V, and VI, with serotype III being the most prevalent (36.1%, 26/72), followed by V (25.0%, 18/72) and Ia (15.3%, 11/72). No isolates were identified for serotype IV. Detailed serotype distributions are shown in [Table 3](#).

In the MLST typing analysis, 72 Group GBS isolates were assigned to 22 distinct sequence types (STs) and further grouped into seven clonal complexes (CCs); unknown clonal complexes are denoted as “Unk.” in subsequent figures. Five isolates could not be assigned to any known ST and were thus designated as new STs (NT). The most common STs were ST19 (15.3%, 11 isolates) and ST862 (9.2%, 7 isolates), with ST19 predominantly appearing in III and V serotypes and ST862 restricted to III serotypes. Additionally, ST17 and ST23 had six isolates each, playing significant roles in the detected STs, with ST17 and ST23 appearing in III and Ia serotypes, respectively. The distribution is detailed in [Figures 1 and 2](#), and a minimum spanning tree is presented in [Figure 3](#).

Table 3 Serotype Distribution of 72 GBS Isolates

Serotype	Number of Strains (n)	Percentage (%)
Ia*	11	15.3
Ib*	9	12.5
II*	7	9.7
III*	26	36.1
V*	18	25.0
VI*	1	1.4

Notes: *Roman numerals in the legend (Ia, Ib, II, III, V and VI) indicate the distinct serotypes among the isolates.

Abbreviation: n, the number of strains.

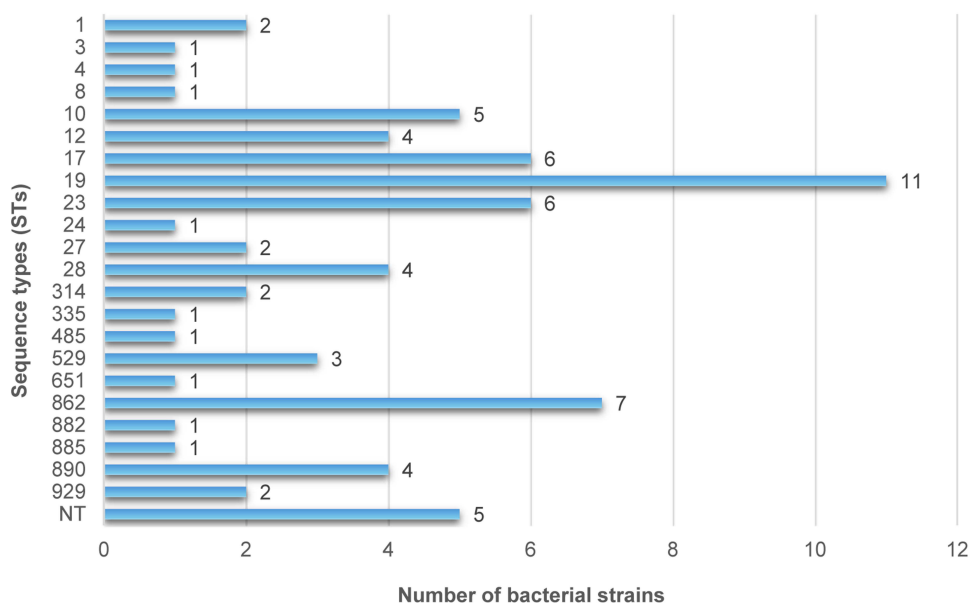


Figure 1 Distribution of GBS strains across sequence types (STs). The horizontal bar chart displays the number of strains on the X-axis and the corresponding STs on the Y-axis. ST19 (n = 11) and ST862 (n = 7) are the most prevalent sequence types, while five strains were non-typeable (NT).

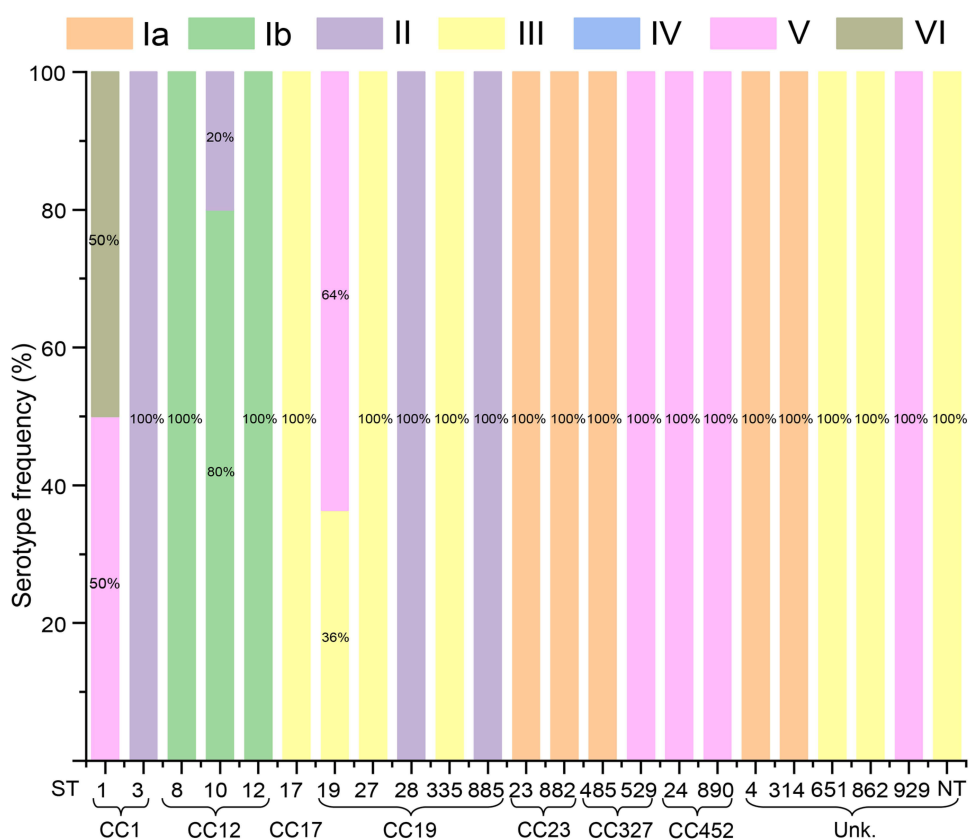


Figure 2 Distribution of serotypes among GBS sequence types (STs), multilocus sequence typing (MLST), and clonal complexes (CCs). The y-axis represents the serotype frequency (%), while the x-axis shows various STs and their associated CCs. Colored bars indicate different serotypes, including Ia, Ib, II, III, IV, V, and VI, with non-typeable (NT) strains also included. The percentage of each serotype within a given ST is labeled on the bars, illustrating the relationship between genetic profiles and serotype distributions.

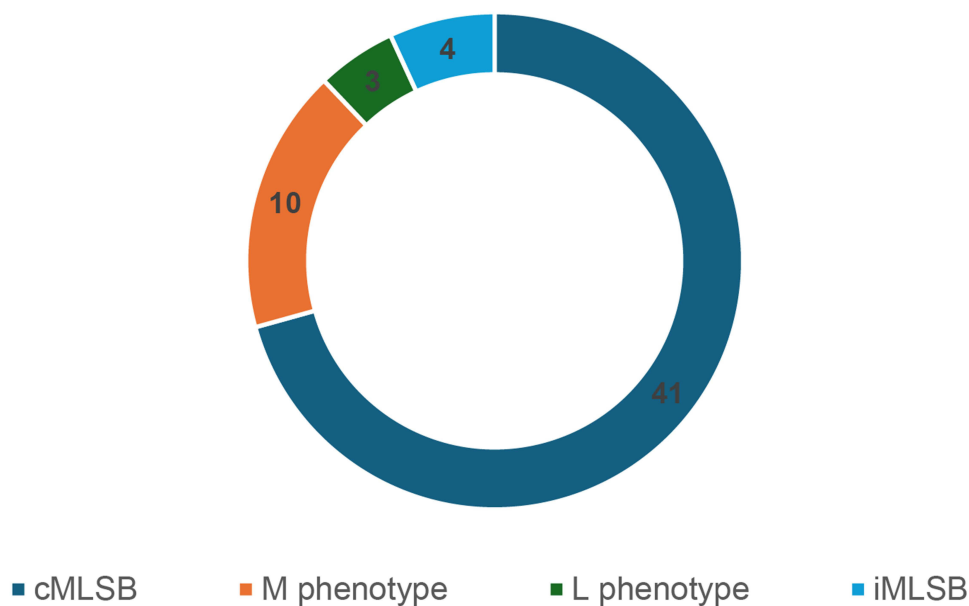


Figure 4 The D-test was performed to detect inducible resistance to clindamycin, showing the distribution of different phenotypes. The cMLS_B phenotype (constitutive MLS_B) exhibits resistance to both clindamycin and erythromycin. The M phenotype is characterized by sensitivity to clindamycin but resistance to erythromycin. The L phenotype demonstrates resistance to clindamycin and sensitivity or intermediate resistance to erythromycin. The iMLS_B phenotype (inducible MLS_B) is defined by resistance to erythromycin and inducible resistance to clindamycin (D-test positive). The donut chart illustrates the quantity and proportion of each phenotype.

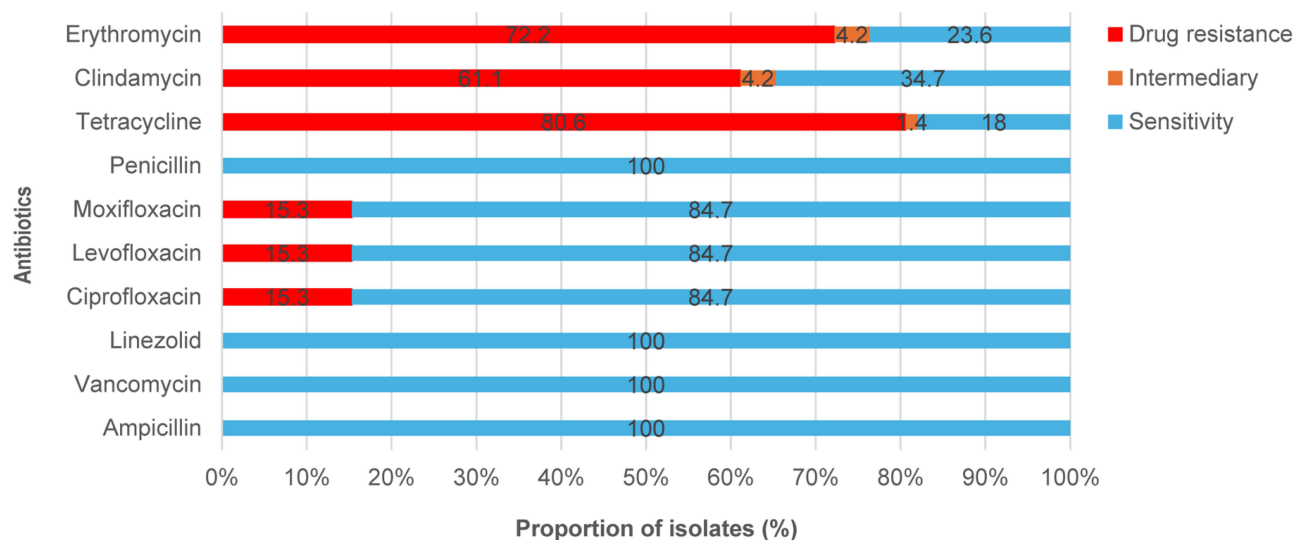


Figure 5 Antibiotic susceptibility results of the tested isolates. The horizontal bar chart shows, for each antimicrobial agent (Y-axis), the proportion of isolates that were resistant, intermediary, or susceptible (X-axis, %). Quinolone antibiotics include levofloxacin, moxifloxacin, and ciprofloxacin. The bar chart represents drug resistance rates (red), intermediary resistance rates (Orange), and susceptibility rates (blue).

Virulence Factors

Based on the cross-distribution of virulence factors by serotype (Figure 6 and Table 5), distinct serotype-specific virulence factor profiles were observed. For example, *lmb*, *bca*, and *scpB* were more commonly detected in serotype III, whereas *cpsJ* and *cpsM* were detected more frequently in serotypes Ia and III, respectively. Overall, serotype III exhibited a broader repertoire of virulence factors, encompassing nearly all factors assessed in this study, indicating that this serotype merits attention in GBS-related clinical contexts. In addition, specific virulence factors such as *hylA* and *hylB* showed differentiated distributions across serotypes.

Table 4 Association of Antibiotic Resistance Genes with Serotypes

Serotype	<i>tetM</i>	<i>tetO</i>	<i>tetL</i>	<i>ermB</i>	<i>mefA/E</i>	<i>ermA</i>	<i>ermTR</i>	<i>lnuB</i>
Ia* (n=11)	9 (81.8%)	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)	0 (0%)	0 (0%)	1 (9.1%)
Ib* (n=9)	1 (11.1%)	4 (44.4%)	0 (0%)	8 (88.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
II* (n=7)	7 (100.0%)	0 (0%)	0 (0%)	1 (14.3%)	4 (57.1%)	1 (14.3%)	1 (14.3%)	0 (0%)
III* (n=26)	14 (53.8%)	10 (38.5%)	1 (3.5%)	13 (50.0%)	3 (11.5%)	2 (7.7%)	2 (7.7%)	8 (30.8%)
V* (n=18)	12 (66.7%)	4 (22.2%)	0 (0%)	4 (22.2%)	6 (33.3%)	5 (27.8%)	5 (27.8%)	2 (11.1%)
VI* (n=1)	1(100.0)	0 (0%)	0 (0%)	1(100.0)	0 (0%)	1(100.0)	1(100.0)	0 (0%)
Total (n=72)	44 (61.1%)	18 (25.0%)	1 (1.4%)	27 (37.5%)	15 (20.8%)	9 (12.5%)	9 (12.5%)	11 (15.3%)

Notes: *Roman numerals in the legend (Ia, Ib, II, III, V and VI) indicate the distinct serotypes among the isolates.

Abbreviation: n, the number of strains.

Discussion

Researching the virulence and antibiotic resistance of GBS provides insight into its pathogenicity and resistance mechanisms.²³ A previous study reported a 9.61% GBS colonization rate among pregnant women in Guangzhou but did not include molecular characteristics such as serotypes, MLST typing, resistance genes, and virulence factors.²⁴ In this report, we investigated the genetic characteristics of GBS-positive isolates collected over six years from a major tertiary hospital in Guangzhou. Using whole-genome sequencing, we analyzed the serotype distribution, sequence types, resistance genes, and virulence factors of GBS isolates. This study contributes to a better understanding of the pathogenicity and antibiotic resistance of GBS, aiding in clinical guidance for GBS infection treatment in the region.

The rich microbial flora in the vagina, with its self-purification capability, and bactericidal factors secreted by the endometrium work together to resist external bacterial infections. However, during pregnancy, women experience a unique immune modulation, entering an immunosuppressed state that protects the embryo by avoiding immune responses against it. This state reduces the mother's immune response to external pathogens, making her more susceptible to bacterial infections.^{25,26} Accordingly, most of the 72 patient strains in this study were derived from pregnant women, with the main sample types being genital secretions and placenta. Both rectal swabs and conjunctival

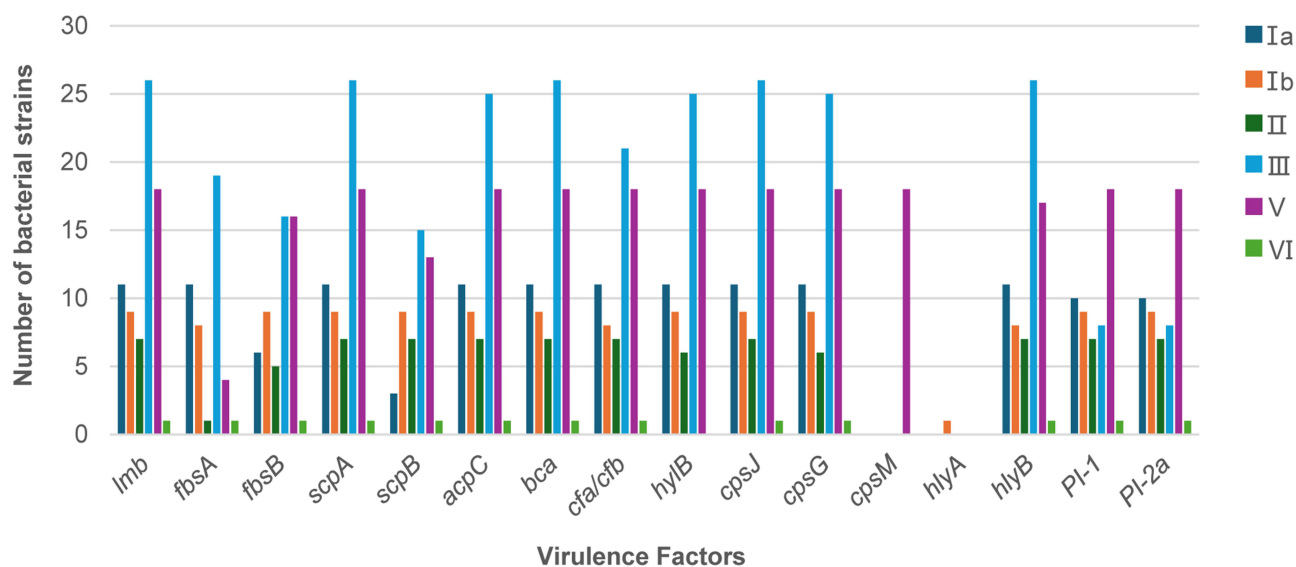


Figure 6 Association between virulence factors and serotypes in 72 GBS strains. The clustered bar chart displays the number of isolates (Y-axis) carrying individual virulence factors for each serotype (X-axis; Ia, Ib, II, III, V, and VI). Different bar colors represent different virulence factors, illustrating their distribution across serotypes.

Table 5 Association of Virulence Factors with Serotypes

Virulence Factor		Total (n=72)	Ia* (n=11)	Ib* (n=9)	II* (n=7)	III* (n=26)	V* (n=18)	VI* (n=1)
Adhesion-related Virulence Factors	<i>lmb</i>	72 (100.0%)	11 (100.0%)	9 (100.0%)	7 (100.0%)	26 (100.0%)	18 (100.0%)	1 (100.0%)
	<i>fbsA</i>	44 (61.1%)	11 (100.0%)	8 (88.9%)	1 (14.3%)	19 (73.1%)	4 (22.2%)	1 (100.0%)
	<i>fbsB</i>	53 (73.6%)	6 (54.5%)	9 (100.0%)	5 (71.4%)	16 (61.5%)	16 (88.9%)	1 (100.0%)
	<i>scpA</i>	72 (100.0%)	11 (100.0%)	9 (100.0%)	7 (100.0%)	26 (100.0%)	18 (100.0%)	1 (100.0%)
	<i>scpB</i>	48 (66.7%)	3 (27.2%)	9 (100.0%)	7 (100.0%)	15 (57.7%)	13 (72.2%)	1 (100.0%)
Escape-related Virulence Factors	<i>acpC</i>	71 (98.6%)	11 (100.0%)	9 (100.0%)	7 (100.0%)	25 (96.2%)	18 (100.0%)	1 (100.0%)
	<i>bca</i>	72 (100.0%)	11 (100.0%)	9 (100.0%)	7 (100.0%)	26 (100.0%)	18 (100.0%)	1 (100.0%)
	<i>cfal/cfb</i>	66 (91.7%)	11 (100.0%)	8 (88.9%)	7 (100.0%)	21 (80.8%)	18 (100.0%)	1 (100.0%)
	<i>hylB</i>	69 (95.8%)	11 (100.0%)	9 (100.0%)	6 (85.7%)	25 (96.2%)	18 (100.0%)	0 (0%)
Escape-related Virulence Factors	<i>cpsJ</i>	72 (100.0%)	11 (100.0%)	9 (100.0%)	7 (100.0%)	26 (100.0%)	18 (100.0%)	1 (100.0%)
	<i>cpsG</i>	70 (97.2%)	11 (100.0%)	9 (100.0%)	6 (85.7%)	25 (96.2%)	18 (100.0%)	1 (100.0%)
	<i>cpsM</i>	18 (25.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	18 (100.0%)	0 (0%)
Hemolysins	<i>hlyA</i>	1 (1.4%)	0 (0%)	1 (100.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	<i>hlyB</i>	70 (97.2%)	11 (100.0%)	8 (88.9%)	7 (100.0%)	26 (100.0%)	17 (94.4%)	1 (100.0%)
Fimbriae	<i>PI-I</i>	53 (73.6%)	10 (90.9%)	9 (100.0%)	7 (100.0%)	8 (30.1%)	18 (100.0%)	1 (100.0%)
	<i>PI-2a</i>	53 (73.6%)	10 (90.9%)	9 (100.0%)	7 (100.0%)	8 (30.1%)	18 (100.0%)	1 (100.0%)

Notes: *Roman numerals in the legend (Ia, Ib, II, III, V and VI) indicate the distinct serotypes among the isolates.

Abbreviation: n, the number of strains.

secretions originated from newborns, totaling 10 cases, accounting for 13.89% of all samples. Research indicates that GBS infections in neonates can lead to early-onset GBS disease.²⁷ In immunocompromised infants, severe GBS infections can be life-threatening and may result in long-term sequelae such as intellectual disability, hydrocephalus, lung damage, and deafness even after recovery.²⁸ This underscores the need for monitoring and treating GBS-positive pregnant women in Guangzhou to prevent vertical transmission and neonatal infections.

GBS has various virulence factors, among which CPS is a key determinant in invasive infections with important epidemiological implications. Anti-CPS antibodies provide protective immunity and are crucial virulence factors for anti-phagocytosis.²⁹ CPS is the main component of the GBS cell wall capsule, with serotypes Ia, Ib, II, and III as the primary strain types, of which serotype III can cause severe infections.^{30,31} This study identified six capsular serotypes, with the most prevalent being serotype III (36.1%, 26/72), followed by serotype V (18 strains, 25.0%) and serotype Ia (11 strains, 15.3%). Studies suggest that serotypes Ia, V, and III are the main serotypes causing invasive infections in neonates,²⁷ while serotypes Ia and V are also prevalent in GBS infections among non-pregnant adults.³² The prevalent serotypes vary slightly by region; for instance, Suzhou has predominant serotypes III, V, and Ib,³³ while Taiwan primarily reports serotypes VI and III.³⁴ The six serotypes detected in this study are consistent with those primarily associated with invasive infections in neonates and non-pregnant adults, highlighting the need for close attention to infections caused by serotypes Ia, Ib, III, and V.

The primary GBS vaccines under development include polysaccharide vaccines covering multiple serotypes and polysaccharide-protein conjugate vaccines.³⁵ The hexavalent conjugate vaccine, covering serotypes Ia, Ib, II, III, VI, and V, and recombinant protein vaccines using alpha-like protein fusion are already in Phase II clinical trials. Regardless of the vaccine type, they aim to provide broad coverage across various strains. This study identified six serotypes, with only one strain of serotype. The hexavalent conjugate vaccine aligns with the current GBS strain prevalence and can cover most pathogenic strains.

This study's GBS isolates were found to contain 22 different ST types, with 5 strains unconfirmed for ST type; these 22 types formed 7 different CCs, with the most common being ST19/V and ST862/III. The distribution of serotypes within CCs also showed clinical diversity in GBS characteristics.^{36–38} Jiang Haoqin and others observed that serotype Ib mainly clustered in ST10 and ST12, while serotype III predominantly clustered in ST19 and ST17. In our study, serotype III was primarily distributed in ST862 and ST17, differing slightly from literature reports, which may be due to differences in GBS serotype prevalence and geographic location. CC17 is the primary clonal complex associated with neonatal GBS meningitis, consistent with reports,³⁹ with six CC17 strains in our study, five of which were from the obstetrics ward. CC17 can acquire new mobile genes through recombination, replacing the original *PI-I* locus, resulting in *PI-I* deletion and carrying resistance genes like *ermB* and *tetO* against erythromycin and clindamycin. This gene acquisition leads to multidrug resistance in the bacteria it harbors.⁴⁰ Additionally, ST23 is recognized as a zoonotic sequence type, initially identified in grey seals.⁴¹ In this study, six GBS isolates were classified as ST23, all of which belonged to serotype Ia. Among the ST23 strains, resistance rates to erythromycin, clindamycin, and tetracycline were 16.7% (1/6), 16.7% (1/6), and 33.3% (2/6), respectively, with erythromycin and clindamycin resistance phenotypes not entirely consistent with the presence of resistance genes. Therefore, investigating the ST types of animal-derived GBS strains in Guangzhou is warranted.

The antibiotic resistance of GBS presents challenges for therapeutic options. Penicillin and ampicillin are the primary drugs for preventing or treating GBS infections, and their susceptibility has remained consistently high;⁴² no strains in this study exhibited resistance to first-line treatments. However, resistance to erythromycin and clindamycin remains high.⁴³ The antimicrobial susceptibility results show that some GBS strains exhibit varying levels of resistance to commonly used antibiotics, possibly due to the widespread presence of this bacterium in clinical environments and the inappropriate use of antibiotics. Additionally, resistance rates varied among isolates. In this cohort, tetracycline resistance was 80.6% (58/72), erythromycin 70.8% (51/72), and clindamycin 61.1% (44/72); all tested isolates were susceptible to penicillin, linezolid, vancomycin, and ampicillin (Figure 4). One study reported a slow decline in GBS resistance rates to erythromycin, clindamycin, and levofloxacin between 2018 and 2020, followed by a slight increase in 2021. Between 2017 and 2021, high resistance rates to tetracycline (75.1%) and erythromycin (65.4%) were observed. Resistance rates to clindamycin and levofloxacin varied significantly, with rates in southern China reported as 55.8% and 24.0%, respectively.⁴⁴ Compared with previous reports, the resistance rates observed in this study were generally lower. Possible explanations include differences in sample composition, time frame, or regional antibiotic-use practices; confirmation will require larger, multi-center datasets. Multidrug resistance was also observed in this cohort: 7.0% (5/72) of isolates were resistant to tetracyclines, clindamycin, erythromycin, and quinolones simultaneously, and 50.0% (36/72) were resistant to erythromycin, clindamycin, and tetracyclines.

Macrolides and related drugs are recommended as alternative therapies for patients allergic to β -lactam antibiotics in GBS infections,⁴⁵ but extensive use of erythromycin and other macrolides could increase resistance risks.⁴⁶ Four GBS isolates in this study exhibited a positive D-test, indicating erythromycin resistance with inducible clindamycin resistance. In clinical management of GBS infections, comprehensive antimicrobial susceptibility testing is essential to avoid the misuse of antibiotics due to empirical treatment. Tetracycline, as a broad-spectrum antibiotic, is less commonly used in GBS infections due to its effects on pregnant women and fetuses, yet it retains high resistance rates. Additionally, serotypes Ib and II showed erythromycin and clindamycin resistance rates of up to 80%, while serotype V demonstrated a tetracycline resistance rate of 90%, suggesting differences in resistance mechanisms may differ across serotypes, leading to varying degrees of resistance to specific antibiotics.

Among resistance genes, the detection rate of the tetracycline-resistance determinant gene *tetM* was 61.1% (44/72), significantly higher than that of the macrolide resistance gene *ermB* (27/72, 37.5%). The resistance profile observed in this study is consistent with recent reports from various regions of China: macrolide and clindamycin resistance remain high, and tetracycline resistance is widespread.⁴⁷ Antibiotic resistance appears to be associated with certain serotypes:⁴⁸ strains carrying *ermB* were mostly of types Ib and III and commonly belonged to CC12/17/19; *mefA/E* was more frequently found in type II; and *tetM* was more often detected in types Ia and II, especially among CC19 strains. The clindamycin resistance gene *lnuB* was primarily found in type III strains. Previous research indicates that *ermB* and *mefA/*

E genes are associated with macrolide resistance.⁴⁹ Continuous monitoring of antimicrobial susceptibility patterns is needed to guide empirical antibiotic therapy.

Virulence genes such as *lmb*, *scpA*, *bca*, and *cpsJ* were found in all 72 strains, corresponding to virulence factors for adhesion, invasion, and immune evasion. Enzymes also represent critical virulence factors in GBS, as they degrade host tissue structures, compromising tissue integrity. The hyaluronidase encoded by *hylB* cleaves hyaluronic acid during infection, promoting GBS dissemination. Mutants lacking *hylB* induce more pro-inflammatory cytokines in vivo and in vitro, resulting in bacterial elimination by the immune system.⁵⁰ The *hylB* detection rate reached 95.8% (69/72), suggesting it facilitates GBS spread. Pili (PI) are also related to pathogenicity, enhancing adhesion to host cells and strengthening GBS invasiveness. Pili play a crucial role in GBS pathogenicity, making them a potential target for GBS vaccine development. The two pilus loci, *PI-1* and *PI-2*, are encoded in different genomic regions; *PI-2* has two variants, *PI-2a* and *PI-2b*, with *PI-2a* most common in human GBS and *PI-2b* more frequent in animal isolates.⁵¹ Studies show that *PI-1* and *PI-2a* are more prevalent in colonizing strains, while *PI-2b* is more likely found in invasive strains. This suggests that GBS strains carrying the *PI-2b* gene have stronger pathogenicity, making them more likely to cause invasive diseases such as septicemia, meningitis, and endocarditis.⁵² In contrast, GBS strains with *PI-1* and *PI-2a* genes are relatively less pathogenic, and *PI-2a* and *PI-2b* do not coexist in the same strain, reducing the likelihood of strains that are both highly pathogenic and colonizing. In this study, 53 strains (73.6%) carried pili, all with *PI-1* and *PI-2a*. Among the six serotypes, only 8 out of 26 strains (30.1%) of serotype III carried pili. As the most common serotype, the low frequency of pili in type III suggests that other virulence factors contribute to GBS colonization.

The hemolysin *hly* can damage host cell membranes, leading to cell death and eliciting host inflammatory responses, further aggravating tissue damage.⁵³ Only one strain of *hlyA* was detected in type Ib; 70 strains (70/72, 97.2%) harbored *hlyB*. All strains demonstrated equal ability in hemolysin-mediated tissue damage, but the unique presence of *hlyA* merits further investigation. Other major virulence factors include cell surface proteins that mediate adhesion and invasion, such as fibrin binding surface protein (*fbsA*), C5a peptidase (*scpA*, *scpB*), which are closely related to GBS adhesion to host cells, epithelial invasion, promotion of platelet aggregation, and anti-phagocytic activities.

This study utilized whole-genome sequencing to evaluate the serotypes, sequence types, resistance genes, and virulence factors of GBS isolates, revealing the prevalent serotypes and sequence types of GBS strains in Guangzhou and analyzing the main resistance genes carried by each serotype. A limitation of this study is its single-center design and relatively small sample size; accordingly, the findings are presented descriptively and no inferential statistics were performed. Nevertheless, these data provide a regional baseline for GBS epidemiology and resistance characteristics and help to orient subsequent investigations. Subsequent studies will expand to establish a multi-center GBS sample repository across major hospitals in Guangzhou, and conduct transcriptomic or proteomic analyses to explore how gene expression changes influence GBS pathogenicity and resistance. In-depth research on GBS is anticipated to clarify why GBS exhibits strong colonization ability but relatively weak pathogenicity, potentially aiding in clinical decision-making for GBS infections in Guangzhou.

Ethical Statement

This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital (Approval No. KY2023-682-01) and was conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants prior to sample collection. All data were de-identified after collection and analyzed anonymously to ensure the protection of participants' privacy and data confidentiality.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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