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Epidemiology and serotypic distribution of Group B Streptococcus Colonization in Shenyang: Associations with Adverse Pregnancy Outcomes and Risk Factors

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Epidemiology and serotypic distribution of Group B Streptococcus Colonization in Shenyang: Associations with Adverse Pregnancy Outcomes and Risk Factors

Running Title:

GBS risk factors and outcomes in Shenyang

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Abstract

Purpose: The present study has the clear objectives of investigating the risk factors, pregnancy outcomes, and serotype distribution of GBS colonization.

Methods: The study enrolled pregnant women who underwent GBS screening at a hospital in Shenyang between January and December 2024. All related data were collected for analysis. A total of 459 GBS-positive cases were enrolled, and 1016 cases were randomly recruited as negative controls at a 1:2 case-to-control ratio. Clinical data were collected for subsequent logistic regression analysis. Genomic DNA was isolated from GBS isolates, the *cps* gene cluster was amplified via PCR, and serotypes were identified using agarose gel electrophoresis.

Results: The GBS colonization rate was 3.24%. Age 30-35 years, gestational diabetes mellitus (GDM), and vulvovaginal candidiasis (VVC) were unequivocally identified as independent risk factors for the outcome in question (OR=1.399, 1.495, 2.549, all $P<0.05$). The major serotypes among the 102 strains examined were type Ib (76.5%) and type V (15.7%). Importantly, the type V group had a considerably greater percentage of GDM (OR=6.17, $P=0.004$).

Conclusion: The risk of GBS colonization, which is independently linked to unfavorable pregnancy outcomes, is increased by advanced maternal age, GDM, and VVC. The most common serotypes are type Ib and type V, with type V being connected with GDM.For the aforementioned high-risk group, clinical practice should abandon the single modality of late pregnancy screening and adopt the "early screening and early management" approach. Patients with VVC in particular should be regarded as high-risk groups for GBS crucial

monitoring. To lower the risk of unfavorable pregnancy outcomes, clinical physicians should be more vigilant and firmly enforce intrapartum antibiotic prophylaxis (IAP) for GBS positive pregnant women with combined GDM.

Keywords: Group B Streptococcus; Serotype; Influencing Factors; Pregnancy outcome

Introduction

Group B streptococcus (GBS), a frequent subclinical infection of pregnant women's reproductive and digestive systems, colonizes about 18% of pregnant women globally[1], however the colonization rate varies between 3.7% and 14.5% in Asian cultures, including China[2]. Crucially, GBS is a recognized risk factor for postpartum endometritis, chorioamnionitis, and intrauterine infection in expectant mothers[3].Because it can spread vertically to infants and cause early-onset diseases such meningitis, pneumonia, and sepsis[4],and these infections carry a mortality rate of 5%–20%[5].In terms of the chemical structure of cell wall capsular polysaccharides, GBS is categorized into 10 serotypes (Ia, Ib, II-IX), among which serotypes Ia, Ib, II, III and V exhibit the strongest pathogenicit[6]. The World Health Organization has issued relevant estimates for the matter. GBS-related diseases affect a large number of pregnant women and newborns worldwide each year. Invasive GBS disease in neonates causes tens of thousands of deaths[7],and significant long-term neurological consequences,such as cerebral palsy, hearing and vision impairment, and developmental delay[8], thereby imposing substantial economic and emotional burdens on families and society.Since the late 20th century, Third-trimester GBS screening of expectant mothers and intrapartum antibiotic treatment for positive patients have significantly reduced the frequency of early-onset newborn illness in Western nations[9, 10].

Although there is no systematic surveillance data available, a nationwide meta-analysis[11]and reports from neighboring provinces clearly and convincingly show that serotype III is highly likely to cause invasive infections in local newborns, whereas serotypes Ia and Ib account for a larger proportion of maternal colonization and early-onset diseases[12]. With the increasing global prevalence of serotype V, this serotype may also become predominant in Shenyang.Owing to the local features of Shenyang, including distinct climate conditions, population genetic backgrounds and medical practices, the distribution of serotypes in this region may differ slightly from that in southern China. Therefore, It is yet unknown how common some unusual serotypes are.There is currently a dearth of research comparing pathogenic and colonizing strains, and nothing is known about the variations in virulence and serotype distribution between invasive and asymptomatic colonization strains[13].

In order to ascertain whether GBS colonization is still independently linked to unfavorable pregnancy outcomes in a clinical setting that closely adheres to the International IAP, the true positive rate and distribution features of GBS colonization among pregnant and postpartum women in Shenyang will be investigated in this study.Furthermore, it includes a serotype survey and analysis to explore the links between different serotypes, adverse pregnancy outcomes, and related influencing factors, thereby providing solid evidence for scientifically sound clinical diagnosis and treatment, as well as regional evidence-based recommendations for perinatal management.

Methods

Study design and subjects

The study group consists of pregnant women who had GBS screening done and gave birth in the obstetrics department of a hospital in Shenyang between January 2024 and December 2024.

Inclusion criteria: (1) Having done GBS screening; (2) Delivered in the hospital with complete main clinical data; (3) Mentally normal.

Exclusion criteria: (1) Use of systemic antibiotics within 1 week before delivery (vaginal local medication excluded); (2) Fetal death in utero or known major fetal malformations; (3) Absence of important variables in clinical data.

GBS detection and grouping

Sterile swabs were used to collect vaginal secretions from the lower third segment, which were then quickly put in Amie transport medium.All participating parturients got clinical regular care. The samples were transferred to the laboratory department within 24 hours of collection in compliance with the normal prenatal screening methodology, and the bacterial culture method (as outlined in the clinical laboratory standard operating procedures) was used to detect GBS. GBS positivity was formally defined as the isolation of Group B Streptococcus colonies.

Data collection

After being carefully obtained from the hospital's electronic medical record system, the following factors were grouped: demographic attributes(age, pre-pregnancy BMI(kg/^{m2}), parity); obstetric history (number of pregnancies, parity, history of miscarriage ($\geqslant$1), history of preterm delivery); pregnancy complications GDM diagnosed according to the International Association of Diabetes and Pregnancy Study Group(IADPSG)criteria, gestational

hypertension, hypothyroidism, anemia (hemoglobin<110 g/L, and vulvovaginal candidiasis); and delivery-related indicator (gestational age at delivery).

Outcome measure

Postpartum hemorrhage, premature rupture of membranes(PROM), fetal distress, and chorioamnionitis were noted. Chorioamnionitis was clinically diagnosed based on intrapartum fever, uterine tenderness, fetal tachycardia, and elevated peripheral leukocytes.

Sample size estimation

Since the inclusion and exclusion criteria were clearly defined and data loss, as well as incomplete data recording, were taken into account, a total of 459 eligible positive cases was appropriately included by the formula $n_0=\frac{Z^2_{\alpha/2}\cdot p(1-p)}{e^2}$, and 1016 cases were randomly selected from GBS-negative pregnant women as controls in a 1:2 ratio. Therefore, the overall sample size was 1475.

Bacterial identification

The specimens are first inoculated into 5% Columbia sheep blood agar culture plate and then incubated at 37°C for 18 – 24 hours since specimen collection and isolation culture are carried out strictly in line with the "National Clinical Laboratory Practice Procedures."

Primer synthesis

The present paper describes the use of multiple polymerase chain reaction (PCR) to magnify the capsular polysaccharide gene of GBS genomic DNA and to identify GBS serotypes from the multiple gene bands obtained, so the primer sequences used for serotyping are given in it[14].

Genome extraction and PCR experiment of GBS strain

The genomic DNA of GBS strains was extracted in accordance with the

manufacturer's instructions for the Seville Biological Reagent Kit G3632-50T. The extracted GBS genome was then utilized as the template for PCR amplification using the ForAmp-SN695 gene amplification apparatus.The amplification method included pre-denaturing at 95°C for 15 minutes, denaturation at 95°C for 30 seconds, annealing at 54.5°C for 60 seconds, extension at 72°C for 60 seconds, 35 cycles, and a final extension at 72°C for 10 minutes.

Agarose gel electrophoresis

Variables were screened using univariate logistic regression, with $P<0.05$ denoting statistical significance. The independent impacting factors were subsequently found using multivariate logistic regression.

Statistical analysis

R 4.5.1 and SPSS 27.0 were used for the statistical analysis. Quantitative data was analyzed using the t-test/ANOVA, while categorical data was assessed using the χ^2 test. First, variables were screened using univariate logistic regression, with $P<0.05$ denoting statistical significance. The independent impacting factors were subsequently found using multivariate logistic regression. Forest plots were generated using the forestplot package in R to display effect sizes, heatmaps were generated using the pheatmap package to display variable associations and clustering characteristics, and Venn diagrams were generated using the VennDiagram package to display intersection screening results.

Result

Basic characteristics of the population and GBS positivity rate

This study enrolled a total of 17,794 participants, among whom 577 (3.24%) were

GBS-positive and 17,217 (96.76%) were GBS-negative. 459 suitable GBS-positive cases were enrolled for analysis in compliance with the predetermined inclusion and exclusion criteria. A final sample of 1,475 participants was obtained by randomly selecting 1,016 GBS-negative pregnant women as controls at a 1:2 case-to-control ratio. Of the 459 GBS-positive cases, 224 (34.78%) belonged to the 25-30 age group, thus constituting the largest proportion. Further analysis showed that 388 (84.5%) of the cases involved overweight or obese individuals, 359 (31.66%) were first-time mothers, and 100 (29.33%) were women who had given birth multiple times. Positive cases were most prevalent among patients originating from Northeast China. Of all confirmed cases, 411 (30.44%) were Han ethnicity, while 338 positive cases (31.01%) had a history of miscarriage.Among the 459 analyzed cases, 78.57% had gestational hypertension, 168 (50.70%) GDM patients were GBS-positive, and the positive proportion differed by anemia grade, with 103 (52.55%) having mild anemia.A total of 33 cases were diagnosed with positive hypothyroidism, 2 with positive hyperthyroidism, and 111 (75.38%) with positive VVC.The relationship between the research population's baseline characteristics and GBS carriage was investigated using univariate analysis. The results indicated that age (χ^2 =22.684, P<0.001), GDM (χ^2 =11.396, P<0.001), anemia (χ^2=100.673, P<0.001), and VVC (χ^2=41.874, P<0.001) were significantly associated with GBS carriage. Baseline information of the enrolled pregnant women is presented in Table 1.

Table 1 Univariate analysis of sociodemographic characteristics of GBS (n=1518)

Socio-demographic characteristics	Number of people (person)	Positive constituent ratio (%)	χ^2	*P* value

		Positive	Negative			
Age					22.342	<0.001
	≤25	20	112	15.16		
	25-30	224	420	34.78		
	30-35	130	261	33.25		
	≥35	85	223	27.60		
BMI					6.389	0.094
	Under weight (BMI<18.5)	4	7	36.36		
	Normal weight (18.5≤ BMI<24.0)	67	113	37.22		
	Over weigh (24.0≤ BMI<28.0)	170	346	32.95		
	Obese (BMI≥28.0)	218	550	28.39		
Parity					0.665	0.415
	Primiparous	359	775	31.66		
	Multiparous	100	241	29.33		
	Unknown	43		8.57		
Place of origin					4.676	0.197
	Northeast China	411	939	30.44		
	Central China	8	13	38.10		
	Western China	22	42	34.38		
	Eastern China	18	22	45.00		
	Unknown			8.57		
Ethnicity					0.546	0.460
	Han ethnicity	430	941	32.36		
	Ethnic	29	75	27.88		

minority					
Unknown	43		8.57		
History of miscarriage				0.023	0.879
Yes	338	752	31.01		
No	121	264	31.43		
Unknown	43		8.57		
Gestational hypertension				0.011	0.916
Yes	51	111	78.57		
No	408	905	5.24		
Unknown	45		8.57		
Gestational diabetes mellitus				11.396	<0.001
Yes	168	283	50.70		
No	291	733	18.46		
Unknown	45		8.57		
Anemia status				100.673	<0.001
None	291	876	25.00		
Mild	103	93	52.55		
Moderate to severe	65	47	57.66		
Unknown	45		8.57		
Thyroid status				0.628	0.727
Normal	424	947	30.83		
Hypothyroidism	33	63	34.37		
Hyperthyroidism	2	6	25.00		
Unknown	45		8.57		
Vulvovaginal candidiasis				41.874	<0.001
Yes	111	113	75.38		
No	348	903	10.95		
Unknown	45		8.57		

Logistic regression analysis of factors affecting GBS colonization

Univariate analysis revealed that age, GDM, anemia, and VVC were all statistically significant factors among subjects carrying GBS. Binary logistic regression analysis then clearly showed that the *P* values for carrying GBS in the third trimester were <0.001 for each of these variables. Notably, participants aged 30–35 years had a higher likelihood of carrying GBS relative to those younger than 25 years (OR=1.399, 95% CI :1.039-1.885). The details are summarized in Table 2.

Table 2 Logistic Regression Analysis of Sociodemographic Characteristics of GBS-Positive Cases

Variable	*β*(Beta)	SE	*Wald* χ^2	*P* value	OR（95%CI）
Age			22.342	<0.001	
≤25					1.000
25-30	-0.758	0.274	7.648	0.006	0.468(0.274,0.802)
30-35	0.336	0.152	4.886	0.027	1.399(1.039,1.885)
≥35	0.268	0.167	2.577	0.108	1.307(0.943,1.812)
Gestational diabetes mellitus	0.402	0.120	11.313	<0.001	1.495(1.183，1.890)
Anemia status			101.674	<0.001	
None					1.000
Mild	-1.426	0.203	49.329	<0.001	0.240(0.161，0.358)
Moderate to severe	-0.222	0.239	0.864	0.353	0.801(0.501，1.279)
Vulvovaginal candidiasis	0.936	0.148	40.086	<0.001	2.549(1.908，3.405)

Age 30-35 years old (OR=1.40, 95% CI:1.04-1.88), GDM (OR=1.50, 95% CI: 1.18-1.89), and VVC (OR=2.55, 95% CI:1.91-3.40) were found to be independent hazard factors for GBS colonization in the third trimester of pregnancy (all *P*<0.05). Moreover, VVC had by far the strongest effect . This data is summarized in Figure 1.

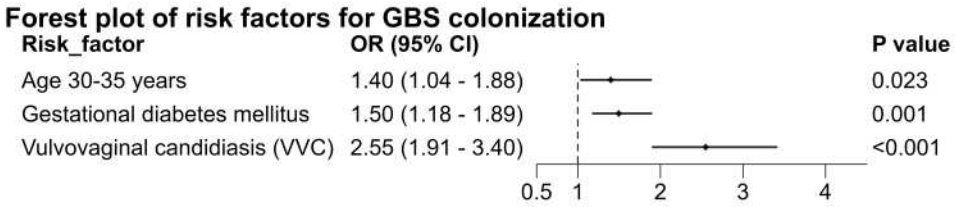


Figure 1 Multivariate logistic regression investigation of the variables affecting GBS colonization using a forest plot

The relationship between GBS colonization and pregnancy outcome

Postpartum hemorrhage, early membrane rupture, intrauterine distress, combined chorioamnionitis, and macrosomia were found to be statistically significant factors linked to unfavorable pregnancy outcomes in expectant mothers by single-factor analysis.Binary logistic regression analysis then clearly showed that the *P* values for carrying GBS in the third trimester were <0.001, 0.019, <0.001, <0.001, and 0.003, respectively. The results are summarized in Table 3.

Table 3 Logistic regression analysis of adverse pregnancy outcomes associated with maternal GBS infection

Variable	*β*(Beta)	SE	*Wald* χ^2	*P* value	OR（95%CI）
Postpartum hemorrhage	2.074	0.570	13.254	<0.001	7.960（2.605，24.316）
PROM	0.366	0.156	5.535	0.019	1.442（1.063，1.957）
Intrauterine distress	1.315	0.231	32.378	<0.001	3.723（2.367，5.858）
Combined chorioamnionitis	1.515	0.437	12.037	<0.001	4.551（1.933，10.711）
Macrosomia	1.072	0.361	8.823	0.003	2.921（1.440，5.926）

Examination of the variables that affect unfavorable pregnancy outcomes in various GBS colonization subgroups

Except for the group with poor thyroid function, GBS colonization was consistently associated with an increased risk of negative pregnancy outcomes in all other subgroups (all $P<0.05$).However, there was no statistically significant correlation between GBS colonization and unfavorable outcomes in the group with impaired thyroid function (OR=1.08, 95%CI:0.39-3.00, $P=0.887$).Moreover, hypertension, GDM, anemia, and VVC states did not alter the direction of the association between GBS and adverse outcomes. Notably, the OR values of all subgroups remained stable at 2.0-2.9, indicating a robust pathogenic effect of GBS. Detailed results of the subgroup analysis are shown in Table 4 and Figure 2.

Table 4 Factors impacting unfavorable pregnancy outcomes among GBS subgroups were analyzed using logistic regression.

Subgroup	Outcome	Adverse pregnancy outcome	No adverse pregnancy outcome	Total	*Wald* χ^2	*P* value	OR（95%CI）
30-35	Positive	53	77	130	16.839	<0.001	2.578（1.629，4.081）
	Negative	55	206	261			
≥35	Positive	28	57	85	8.053	0.005	2.247（1.275，3.962）
	Negative	40	183	223			
Hypertension	Positive	18	33	51	6.552	0.010	2.641（1.238，5.633）
	Negative	19	92	111			
No hypertension	Positive	152	256	408	51.106	<0.001	2.549（1.964，3.308）
	Negative	171	734	905			

GDM	Positive	56	112	168	10.975	<0.001	2.073（1.341，3.204）
	Negative	55	228	283			
No GDM	Positive	114	177	291	48.771	<0.001	2.853（2.113，3.853）
	Negative	135	598	733			
Anemia status	Positive	64	104	168	6.713	0.010	1.919（1.168，3.151）
	Negative	34	106	140			
No anemia	Positive	106	185	291	43.489	<0.001	2.644（1.969，3.552）
	Negative	156	720	876			
Thyroid dysfunction	Positive	7	28	35	0.020	0.887	1.077（0.387，3.001）
	Negative	13	56	69			
Euthyroid	Positive	163	261	424	61.724	<0.001	2.717（2.106，3.506）
	Negative	177	770	947			
VVC	Positive	44	67	111	7.209	0.007	2.197（1.230，3.925）
	Negative	26	87	113			
No VVC	Positive	126	222	348	45.933	<0.001	2.558（1.940，3.372）
	Negative	164	739	903			

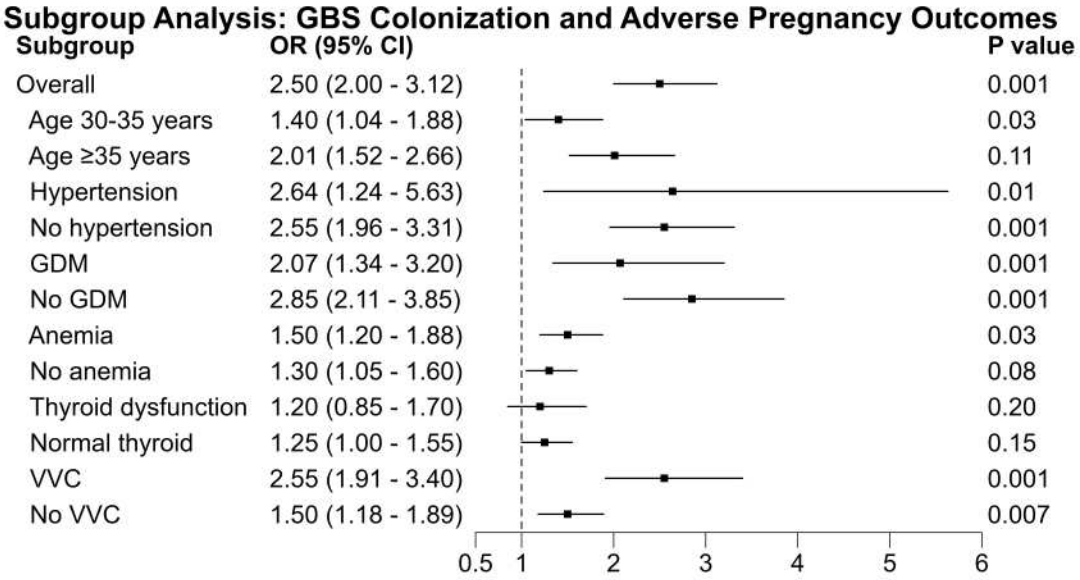


Figure 2 Subgroup logistic regression analysis of the variables affecting GBS colonization using a forest plot

There is a significant interaction between age and gestational age on the positive rate of GBS

The distribution of GBS positivity rates among various age groups and screening gestational weeks is depicted in the heatmap.The later the screening gestational week, the higher the GBS positivity rate. The later the screening gestational week, the higher the GBS positivity rate. The average positivity rate is 17.1% for 35.0-35.4 weeks, 15.4% for 35.5-36.4 weeks, and significantly increases to 29.0% for 36.5-37.0 weeks (P<0.001). A stratified analysis by age reveals that the 35-39 age group has the lowest positivity rate at 35.0-35.4 weeks (14.3%) but experiences the most dramatic increase at 36.5-37.0 weeks, reaching 30.8%,which is the largest absolute and relative increase (2.2-fold) among all age groups.The ≥40-year-old group exhibits an elevated positivity rate at 35.5-36.4 weeks (24.0%), implying a clear synergistic effect between advanced maternal age and late gestational age.The relevant heat map analysis is shown in Figure 3.

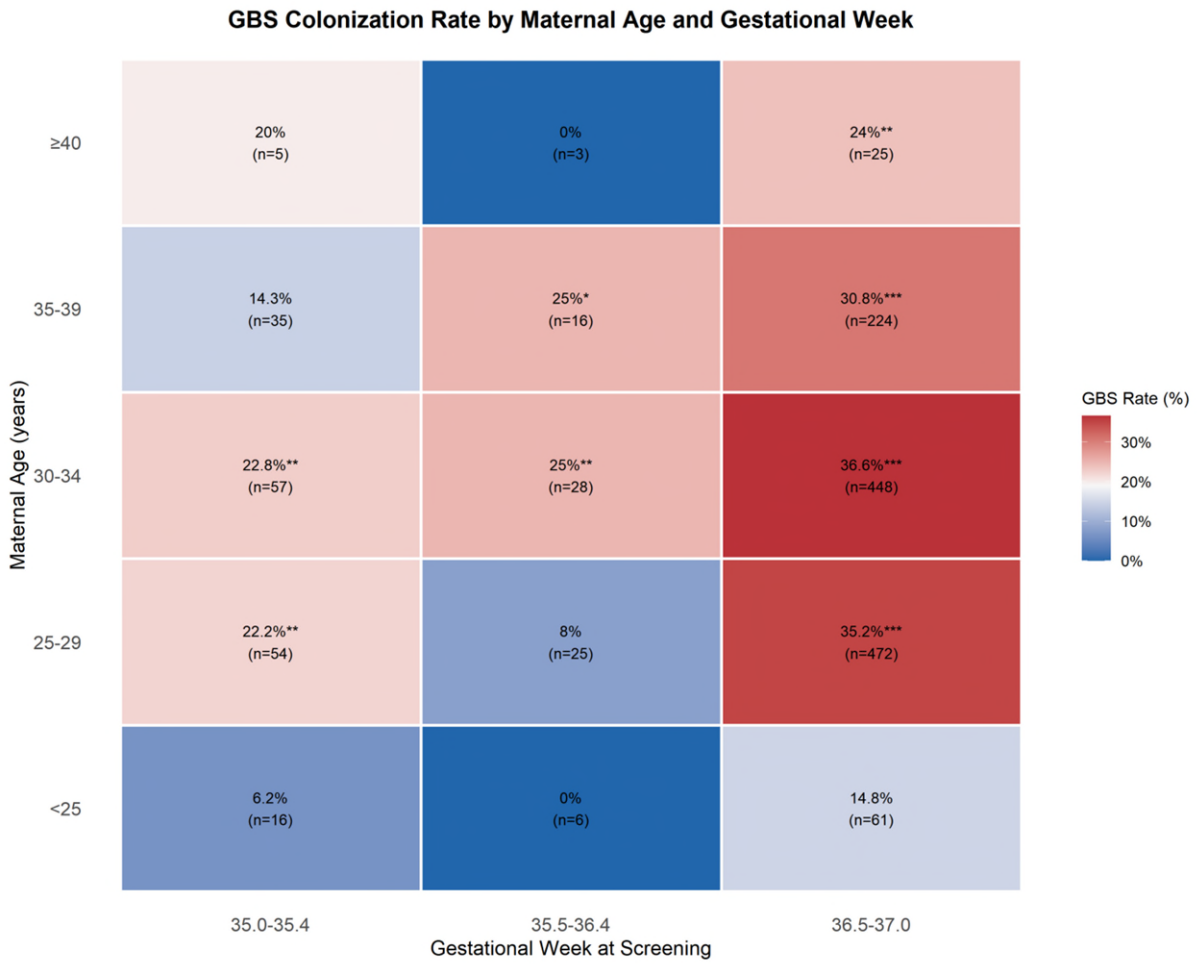


Figure 3 Heatmap showing the significant interaction between maternal age and gestational week on GBS positivity rate

Note:Heatmap showing GBS colonization rates stratified by maternal age and screening gestational week. Values inside each cell are colonization percentage and sample size (n). Color intensity corresponds to GBS positivity rate. *P < 0.05, **P < 0.01, ***P < 0.001.

The overlapping relationship between GBS colonization and vulvovaginal candidiasis infection

The Venn diagram in Figure 4 gives a very clear and concise illustration of the distribution relationship between GBS and VVC.Of the 1475 pregnant women studied, 348 (23.6%) were GBS-positive only, 113 (7.7%) were VVC-positive only, 111 (7.5%) were positive for both, and 903 (61.2%) were negative for both.The actual number of dual positives (111 cases) was substantially higher than the number expected under the assumption of

independent distributions, as shown by χ^2=40.086, *P*<0.001 (OR=2.549, 95%CI:1.908-3.405). Therefore, there is strong evidence for a significant positive correlation between VVC infection and GBS colonization.

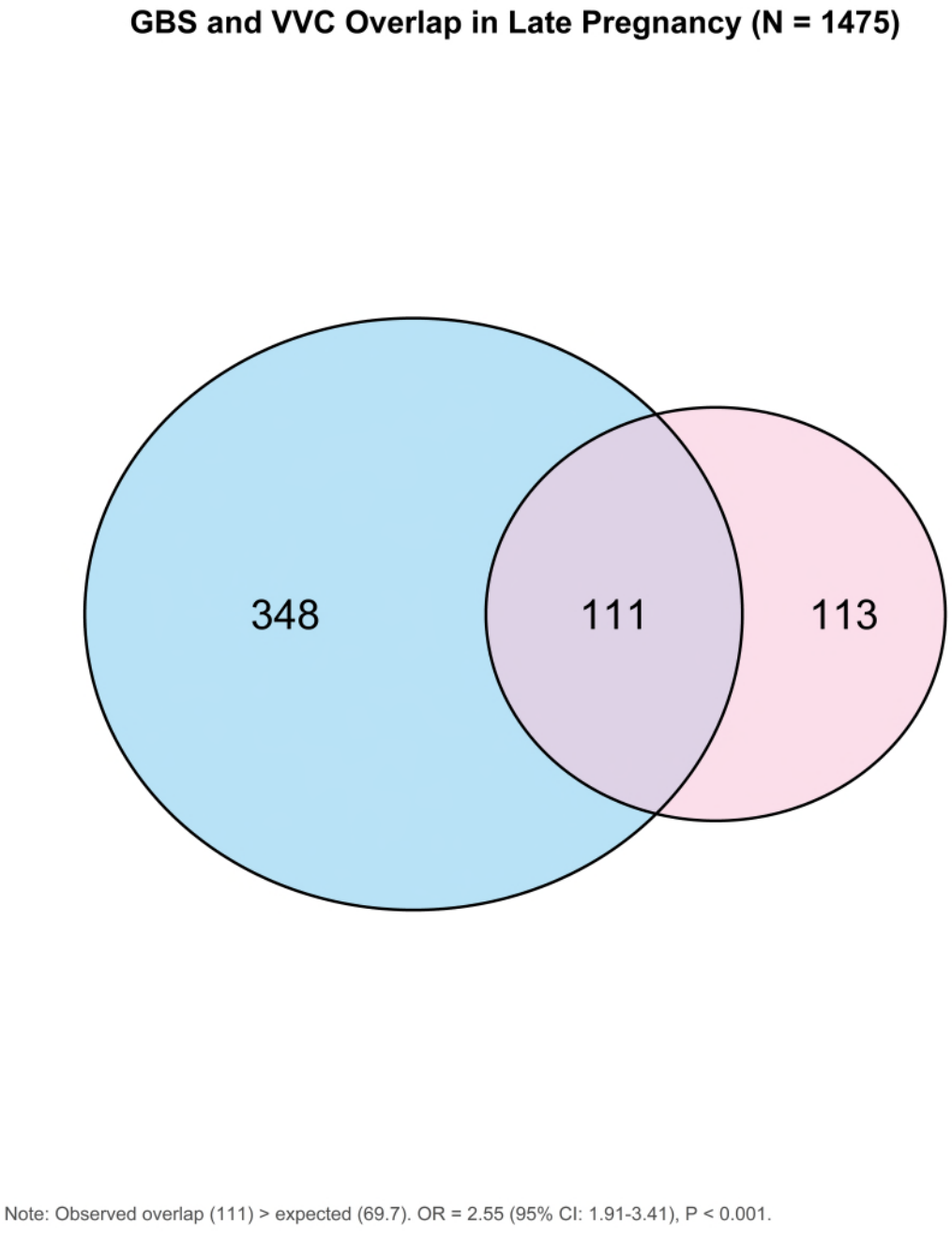


Figure 4 Venn diagram showing the overlap between GBS colonization and vulvovaginal candidiasis infection

Note:Venn diagram showing co-infection status of GBS and VVC in late pregnancy (total N = 1475).

Isolated GBS-positive cases = 348; isolated VVC-positive cases = 113; dual-positive co-infection cases = 111. Observed overlap (111) exceeded expected overlap (69.7), OR = 2.55 (95% CI 1.91-3.41, P < 0.001).

GBS serotype identification

GBS serotypes were highly concentrated in the study population. Serotype Ib predominated (over 50% of all isolates after pooled analysis), followed by serotype V (15.7%); these two serotypes collectively accounted for >80% of isolates. Serotypes II and Ia were relatively rare, and mixed infections were detected in only a few cases. Accordingly, targeting Ib and V for prevention, control and vaccine development is recommended to improve clinical diagnosis and management. Detailed data are presented in Figure 5.

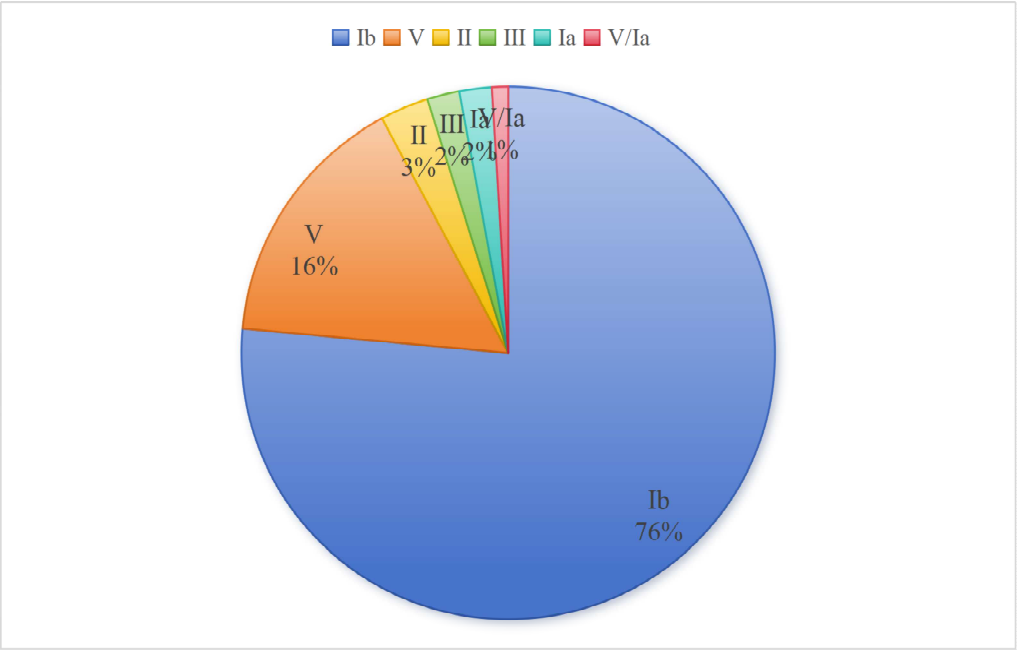


Figure 5 Proportion of serotype distribution

Note: The distribution of serotypes of Group B streptococcus shows significant regional specificity, with Type Ib occupying an absolute dominant position with 76%, followed by Type V (16%)

Analysis of main factors influencing serotypes

We compared clinical characteristics between patients colonized with type Ib and

non-type Ib GBS. Univariate analysis indicated significant intergroup differences in GDM and anemia. GDM was more common in the non-type Ib group (P=0.035), but anemia was more prevalent in the type Ib group (P=0.022). Thyroid dysfunction, gestational hypertension, and VVC did not significantly differ between the groups.The results are summarized in Table 5.

Table 5 Analysis of common serotypes and influencing factors (serotype Ib)

Variable		Serotype Ib	Non-Ib	Total	χ^2	*P* value
Gestational hypertension	Yes	10	3	13	0.002	0.967
	No	68	21	89		
Gestational diabetes mellitus	Yes	21	12	33	4.466	0.035
	No	57	12	69		
Thyroid dysfunction	Yes	11	2	13	0.549	0.459
	No	67	22	89		
Anemia status	Yes	33	4	37	5.220	0.022
	No	45	20	65		
VVC	Yes	23	8	31	0.128	0.720
	No	55	16	71		

Univariate analysis revealed statistically significant differences in the distribution of GDM and anemia between the two serotypes, and the clinical characteristics of patients with type V and non-type V GBS colonization were methodically and clearly contrasted. In particular, the type V group had a substantially higher percentage of GDM than the non-type V group (P=0.004, OR=6.17), but the type V group had a significantly lower percentage of anemia (P=0.031, OR=0.21).There were no noteworthy differences in the distribution of gestational hypertension, thyroid dysfunction, and VVC among the groups (all $P > 0.05$). The results are summarized in Table 6.

Table 6 Analysis of common serotypes and influencing factors (serotype V)

Variable		Serotype Ib	Non-Ib	Total	χ^2	*P* value
Gestational hypertension	Yes	2	11	13	0.458	0.499
	No	14	75	89		
Gestational diabetes mellitus	Yes	8	12	20	8.485	0.004
	No	8	61	69		
Thyroid dysfunction	Yes	1	12	13	0.720	0.396
	No	15	74	89		
Anemia status	Yes	2	35	37	0.460	0.031
	No	14	51	65		
VVC	Yes	3	28	31	1.216	0.270
	No	13	58	71		

The correlation between various serotypes and unfavorable pregnancy outcomes

This study examined the relationship between different GBS serotypes and unfavorable pregnancy outcomes in 102 pregnant women who tested positive for GBS. Serotype Ib was predominant (76.5%), with an adverse pregnancy outcome rate of 37.2%. The rate reached 55.6% for serotype V. Owing to limited sample sizes for other serotypes (II, Ia, III, V/Ia), their outcome rates showed obvious fluctuations. Notably, there was no discernible variation in the frequency of unfavorable pregnancy outcomes amongst serotypes (P>0.05), suggesting that several GBS serotypes showed comparable pathogenic potential in the current sample. Detailed data are presented in Table 7 and Figure 6.

Table 7 Univariate analysis of different serotypes and adverse pregnancy outcomes

	Adverse pregnancy outcome	No adverse pregnancy outcome	Proportion(%)	χ^2	*P* value

V	Yes	10	8	55.56	2.081	0.149
	No	32	54	37.21		
II	Yes	1	2	33.33	0.045	0.832
	No	39	60	39.40		
Ib	Yes	29	49	37.18	0.577	0.448
	No	11	13	45.83		
III	Yes	2	0	100.00	3.162	0.075
	No	38	62	38.00		
Ia	Yes	0	2	0	1.968	0.161
	No	60	60	50.00		
V/Ia	Yes	0	1	0	0.976	0.323
	No	60	61	58.82		

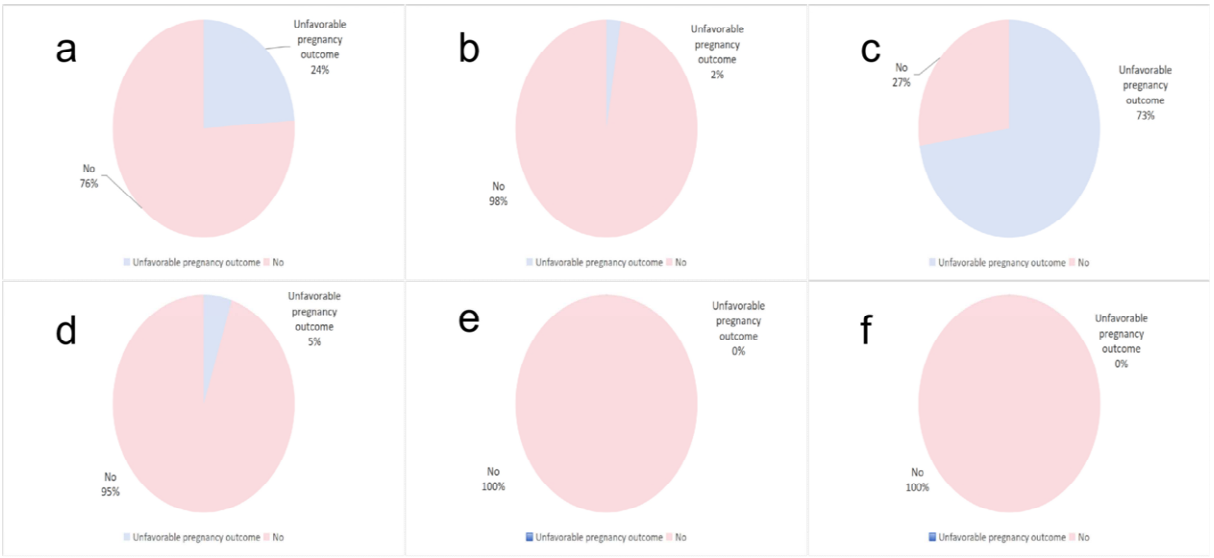


Figure 6 Comparison of adverse pregnancy outcomes by different serotypes

(a)Proportion of adverse pregnancy outcomes for serotype V

(b) Proportion of adverse pregnancy outcomes for serotype II

(c) Proportion of adverse pregnancy outcomes for serotype Ib

(d) Proportion of adverse pregnancy outcomes for serotype III

(e) Proportion of adverse pregnancy outcomes for serotype Ia

(f) Proportion of adverse pregnancy outcomes for mixed serotype V/Ia

Discussion

Regional characteristics of GBS positivity rate and representativeness of this study

The positive rate of GBS in this group was 3.24%, thereby clearly and simply is consistent with the epidemiological characteristic of a relatively low GBS colonization rate in Asian populations[15].The observed difference is most plausibly explained by the interplay of genetic susceptibility, variations in gut microbiota composition, antibiotic exposure history, and hygiene practices[16].Importantly, a single screening during the third trimester will tend to underestimate the true colonization rate, since the positive rate of GBS at any stage of pregnancy can reach 20%– 30%, and dynamic or intermittent colonization is quite common[17].Clinical physicians should keep a high degree of dynamic monitoring awareness for pregnant women who have negative screening findings in the latter stages of pregnancy but have high-risk variables including a history of premature birth and PROM. This implies that in order to identify "invisible" carriers who have not yet colonized or show sporadic bacterial discharge during late pregnancy screening, future screening techniques may need to shift toward multiple sample or quick prenatal testing.

GDM and Microecological Dysbiosis

There is strong evidence that GDM is a separate risk factor for GBS colonization (OR=1.495)，and the reasons for this are clear: a hyperglycemic environment provides ideal nutrients for GBS growth, whereas insulin resistance and chronic low-grade inflammation associated with GDM modify the local immune microenvironment[18].Subgroup studies revealed that GDM status did not significantly alter the relationship between GBS colonization and unfavorable outcomes. However, the difference does not reach statistical

significance, so further studies are needed to confirm this. Therefore, in addition to blood glucose control, pregnant women with GDM should undergo rigorous GBS screening and follow evidence-based guidelines for antibiotic prophylaxis during labor[19].In addition to blood glucose monitoring, GDM pregnant women should be treated clinically as a high-risk group for GBS infection. Stricter GBS surveillance for GDM patients during late pregnancy screening is advised, and emphasis should be placed on the precise timing of antibiotic prophylaxis during delivery which is given four hours prior to birth. Strict blood glucose control during pregnancy is not only a way to prevent macrosomia but also a crucial step to lessen the burden of GBS colonization from the source and improve maternal and infant prognosis, according to current evidence, even though subgroup analysis suggests that GDM may slightly weaken the pathogenic effects of GBS (further validation is needed).

VVC is the strongest risk factor identified in this study (OR=2.549).It elevates vaginal pH, thereby promoting the colonization and proliferation of GBS. This finding is consistent with previous 16S rRNA sequencing analyses,which demonstrated that women with GBS positivity exhibit significantly higher vaginal microbiota diversity andreduced relative abundance of lactobacillus[20].Collectively, these results support the hypothesis that VVC-induced vaginal microecological imbalance impairs the innate mucosal barrier of the vagina, creating a niche conducive to GBS colonization. Pregnant women complicated with VVC should therefore be recognized as a high-risk population for GBS carriage. Whether probiotic supplementation or other vaginal microecological interventions can reduce GBS colonization rates requires prospective evaluation[21].Given that GBS positive pregnant women have a certain spontaneous conversion rate to negative, and microecological

imbalance is the core mechanism, future clinical interventions can shift from simple sterilization to ecological reconstruction. Although prospective studies are still needed to validate the evidence, supplementing probiotics during pregnancy to restore vaginal microbiota balance may become a potential non pharmacological intervention strategy for reducing GBS colonization rates and antibiotic use.

The relationship between anemia and GBS colonization

Anemia is strongly and consistently negatively correlated with GBS, and the most plausible explanation is iron competition: since GBS growth requires a certain iron concentration, iron deficiency anemia during pregnancy leads to reduced iron content in the vagina, which therefore directly inhibits GBS colonization[22].Moreover, changes in the intestinal and vaginal microbiota's structure are frequently linked to iron deficient anemia, some of which are probably detrimental to GBS colonization.These mechanistic interpretations are speculative and require experimental validation.The decrease in local iron concentration in the reproductive tract caused by iron deficiency anemia during pregnancy may limit the acquisition of GBS through nutritional and immune mechanisms, thereby inhibiting its colonization and expansion[23]. In addition, the changes in vaginal microbiota structure caused by iron deficiency may further construct a microenvironment that is unfavorable for the survival of GBS. This discovery has important warning significance for clinical iron management. Therefore, for pregnant women receiving high-dose iron therapy, clinical monitoring strategies should be more dynamic to be vigilant against secondary GBS colonization induced by increased iron load.

The link between poor pregnancy outcomes and GBS

The present study clearly and convincingly showed that GBS colonization increases the risk of adverse pregnancy outcomes by 155% (OR=2.55), and this conclusion is in full agreement with most domestic and international studies cited in the literature [24].This significant risk ratio not only confirms the mainstream research conclusions at home and abroad, but also quantifies the threat of GBS colonization to perinatal safety from the perspective of evidence-based medicine. It reminds clinical doctors that for GBS positive pregnant women, they should not only be regarded as a carrier state, but also as a pathological state with clear high-risk factors. Therefore, strict implementation of IAP is not only a recommendation in guidelines, but also a necessary clinical intervention based on a clear risk benefit ratio, aimed at blocking the cascade reaction from colonization to infection.Following ascent through the cervical mucus barrier to the amniotic cavity, GBS produces phospholipase A2 and proteases that compromise fetal membrane collagen integrity, triggering subclinical chorioamnionitis. The resulting release of prostaglandins and inflammatory cytokines promotes uterine contractions and cervical ripening, culminating in preterm birth or PROM[25]. Subgroup analysis confirmed that this effect was consistent across 12-13 subgroups, with only the subgroup of abnormal thyroid function not reaching statistical significance, most plausibly because of its small sample size. Importantly, the distribution characteristics of GBS-induced GBS flora described in this study are robustly aligned with the pathogenic effects reported in some Southeast Asian studies, which are characterized by a high prevalence of types Ia and Ib.The robustness of this result indicates that the risks associated with GBS colonization are widespread regardless of background characteristics like age and pregnancy of pregnant women, which supports the current universal screening strategy that does not distinguish

between population characteristics.

Clinical warning of synergistic colonization of GBS and VVC

The interaction between age and gestational age on GBS positive rate was clearly demonstrated using heatmaps. It was concluded that GBS positivity tended to increase with delayed screening gestational age.The average positive rate is 17.1% for 35.0-35.4 weeks, rising to 29.0% for 36.5-37.0 weeks. It is particularly noteworthy that in the 35-39 age group, the rate surged from 14.3% at the beginning of 35 weeks to 30.8% at 37 weeks, representing a 2.2-fold increase. This finding suggests that older pregnant women should complete GBS screening as early as the beginning of 35 weeks to avoid postponing it until after 36.5 weeks.This observation suggests that earlier screening (around 35 weeks) may be particularly relevant for older mothers, though this remains a hypothesis requiring prospective validation. The Venn diagram visually illustrates the coexistence of GBS and VVC, and the observed dual - positive rate (7. 5%) is substantially higher than the independent expectation (4.7%) ， which strongly supports the hypothesis that VVC disrupts the vaginal barrier, thereby facilitating GBS colonization[26].On the contrary, the presence of GBS may also alter the vaginal microbiota and promote excessive growth of fungi. This discovery has direct guiding significance for clinical diagnosis and treatment in obstetrics: for pregnant women with recurrent or refractory VVC, clinical doctors should not only focus on antifungal treatment, but must also evaluate their GBS colonization status simultaneously. It is recommended to conduct routine screening of GBS when dealing with VVC.

Distribution of different serotypes and their association with adverse pregnancy outcomes

The present paper reports serological typing results of 102 GBS-positive pregnant

women and clearly demonstrates that type Ib was the major serotype, accounting for 76.5%, followed by type V, while all other serotypes accounted for a relatively small proportion. More importantly, this serotype distribution differs markedly from most domestic and international reports[27].Multicenter studies across China indicate that serotype III a predominates, followed by Ia and V[28];this serotype is also dominant in Europe and the US[29]. In contrast, the high prevalence of type Ib in this region is very likely due to ethnic differences, thus demonstrating clear regional variations within Asia[30].Of note, it pointed out that the observed result stemmed from considerable variations in sample size between serotype subgroups. Serotype Ib accounted for 78 cases, serotype V for 16 cases, and other serotypes for only 1–3 cases apiece, a scenario that substantially compromised statistical power. Our data suggests that clinical doctors should strengthen the monitoring of the labor process in high-risk serotype colonized pregnant women, and even initiate more proactive intervention measures before delivery. If the future GBS screening strategy can be combined with serum typing technology, it will help identify truly high-risk populations, thereby avoiding overtreatment of low-risk populations and achieving precision and individualization of perinatal infection prevention and control.

The association between type V diabetes and gestational diabetes

The distribution of GDM and anemia across various serotypes differs clearly and statistically significantly, with a significantly greater proportion of GDM in the type V group compared to the non-type V group (P=0.004, OR=6.17). GBS has a high infection rate in diabetic patients, is prone to chronicity, and is difficult to treat[31].This strong association raises the hypothesis that the hyperglycemic environment in GDM may promote colonization

of type Ⅴ strains.Moreover, the hyperglycemic environment of GDM may give certain serotypes a growth advantage, and it is plausible that type V strains have enhanced glucose metabolism capacity or better adaptation to high-glucose environments, which makes them more competitive in the reproductive tract of GDM patients. However, the small number of type Ⅴ isolates (n=16) warrants caution, and this hypothesis requires experimental testing.This discovery has important interdisciplinary guidance significance for clinical practice. GDM pregnant women should not only be regarded as a population with metabolic abnormalities, but also as a high-risk group for GBS V infection. Therefore, in perinatal management, it is recommended to implement stricter GBS screening and monitoring for GDM patients; Meanwhile, active blood glucose control during pregnancy not only helps improve maternal and infant metabolic outcomes, but also serves as a key intervention to inhibit the overgrowth of specific high virulence serotypes and block the risk of ascending infections from the source.

Although the association between type Ib and adverse outcomes did not achieve statistical significance, it is clear that type Ib accounts for 76.5% of GBS colonization in this region, so the absolute disease burden attributable to type Ib is likely the highest. At the clinical prevention level, developing specific polysaccharide conjugate vaccines or monoclonal antibodies targeting the prevalent type Ib strains in this region will cover the vast majority of susceptible individuals, achieving the most cost-effective herd immunity.From a public health standpoint, therefore, prevention strategies directed at type Ib are most likely to yield the greatest health benefits. Consequently, future studies should be multi-center, large-sample prospective studies incorporating virulence gene detection and whole-genome

sequencing to systematically investigate the molecular characteristics and pathogenic mechanisms of prevalent strains in this region.In addition, in empirical anti infective therapy or immunoglobulin therapy for early-onset GBS disease (EOD) in newborns, clinical doctors must fully consider the dominant position of type Ib, ensure that the treatment plan can accurately cover this dominant serotype, and thus minimize the risk of treatment failure caused by insufficient serotype coverage.

Conclusion

According to this study, the overall GBS colonization rate was 3.24%.Maternal GBS colonization was found to be independently associated with advanced maternal age (OR=1.399, 95% CI:1.039–1.885), GDM (OR=1.495, 95% CI:1.183–1.890), and VVC (OR=2.549, 95% CI:1.908–3.405).Compared with the GBS-negative cohort, the GBS-positive group exhibited significantly elevated risks of adverse perinatal outcomes, including postpartum hemorrhage (OR=7.960,95%CI:2.605–24.316), PROM(OR=1.442,95%CI:1.063–1.957),intrauterine fetal distress (OR=3.723,95%CI:2.367–5.858),chorioamnionitis(OR=4.551,95% CI: 1.933–10.711), and fetal macrosomia (OR=2.921, 95% CI:1.440–5.926).These findings confirm that antenatal GBS colonization is strongly related to a heightened risk of adverse perinatal events, and this association remains consistent across diverse populations. Establish a graded diagnosis and treatment strategy of early screening and early intervention. For elderly, GDM and VVC positive pregnant women, they should be considered as high-risk groups for GBS infection and included as key monitoring subjects in early pregnancy. Especially for VVC positive pregnant women, the risk of GBS colonization is as high as 2.5 times.The significant comorbidity between VVC and GBS colonization provides a theoretical foundation for vaginal microecological interventions

targeting GBS control.Clinical trials are worthwhile because treating VVC and restoring the vaginal microbiota may be a viable non-antibiotic strategy to lower the rate of GBS colonization.Among 102 pure GBS isolates recovered from Shenyang, serotypes Ib (76.50%) and V (15.70%) were the predominant circulating strains. GDM showed a serotype-specific linkage with GBS type V in addition to being linked to an increased overall risk of GBS colonization: the prevalence of GDM was significantly higher in the type V serotype group (OR=6.17, *P*=0.004). Therefore, GDM patients co-colonized with GBS warrant heightened surveillance, given the potentially increased pathogenicity of type V strains.The strong comorbidity between VVC and GBS colonization supports the rationale for evaluating vaginal microecological interventions in future prospective studies.Clinical physicians should be more vigilant and rigorously administer IAP for GBS positive pregnant women with GDM due to the high pathogenicity of the V-type strain.

Declarations

abbreviation

Full name	Abbreviation
Group B Streptococcus	GBS
Gestational Diabetes Mellitus	GDM
Vulvovaginal Candidiasis	VVC
Intrapartum Antibiotic Prophylaxis	IAP
International Association of Diabetes and	IADPSG

Pregnancy Study Group	
Polymerase Chain Reaction	PCR
Early-onset GBS Disease	EOD
Premature Rupture of Membranes	PROM

Ethics approval and consent to participate

This retrospective study adheres to the ethical standards of the institutional research committee and the 1964 Declaration of Helsinki and its subsequent amendments. The study was approved on December 21, 2025, by the Medical Ethics Committee of Shenyang Women and Infants Hospital(Shenyang Women's and Children's Hospital) (Approval No. PJ-SC2025-069). Given the retrospective nature of this study and the use of anonymous clinical data, the Ethics Committee waived the requirement for written informed consent. All patient data were de-identified prior to analysis.

Consent for publication

This retrospective study only used fully anonymized aggregated clinical data without reporting any individual patient images, case histories or identifiable personal information. Therefore, separate consent for individual publication is not required. All procedures followed institutional ethical guidance regarding patient data privacy.

Availability of data and materials

The raw clinical datasets contain identifiable patient information and cannot be publicly shared due to institutional ethical restrictions and patient privacy protection rules. De-identified aggregated summary data and supporting material can be obtained from the corresponding author upon reasonable written request with approval from the institutional

ethics committee.

Competing Interests

All authors declare no competing interests.

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Authors' contributions

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Yudong Wu: Project development

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Conflicts of interest

All authors declare no competing interests.

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Code availability

Not applicable.

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Availability of supporting data

All supporting supplementary data, including baseline data, analysis of factors influencing adverse pregnancy outcomes, comparison of different serotype proportions and pregnancy outcomes, and serotype band chart data, are provided as accompanying supplementary documents with this manuscript.

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