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DISTRIBUTION OF GROUP B STREPTOCOCCUS SEROTYPE III AMONG PREGNANT WOMEN IN HANOI

Nguyen Le Van[1], Nguyen Thi Nhu Quynh[2],
Ha Thi Thu Van[1], Nguyen Hung Cuong[1], Hoang Xuan Quang[1], Do Ngoc Anh[2*]

[1]*Military Hospital 103 - 261 Phung Hung, Phuc La Ward, Ha Dong District, Hanoi City, Vietnam*
[2]*Vietnam Military Medical Academy - 160 Phung Hung, Phuc La Ward, Ha Dong District, Hanoi City, Vietnam*

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ABSTRACT

Objectives: We investigated the distribution of Group B Streptococcus (GBS) serotype III among pregnant women in Hanoi, and its association with other factors.

Methods: A cross-sectional study was conducted on 876 pregnant women at a gestational age of 35–37 weeks in three hospitals in Hanoi, Vietnam from October 2021 to May 2022. Vaginal-rectal samples were collected to screen GBS using culture and PCR methods. PCR techniques were also used to analyze the serotypes of GBS strains. The data were analyzed to determine the prevalence of serotype III and its relationship to other factors.

Results: Of the 171 GBS isolates, 40 strains (23.39%) were identified as serotype III, accounting for the highest prevalence. The age group of 30 to under 35 years had the largest number of serotype III infections. Pregnant women infected with serotype III (32.5%) had a slightly higher prevalence of genitourinary tract infections (GTIs) history than those infected with other serotypes (28.24%).

Conclusion: Serotype III is the predominant GBS serotype among GBS isolates from pregnant women in Hanoi. Further research is needed to explore the immune response mechanisms and the impact of serotype III on maternal and neonatal outcomes.

Keywords: Group B Streptococcus, serotype III.

1. INTRODUCTION

Group B Streptococcus (Streptococcus agalactiae) is one of the leading causes of morbidity and mortality in newborns. Invasive infection usually manifests as early-onset disease in the first week of birth. GBS is transmitted from mother to child during labor and rupture of membranes, the fetus will swallow or inhale GBS-contaminated amniotic fluid. Different investigations have revealed that the prevalence of GBS infection in pregnant women at 35 - 37 weeks is from 7.1% to 35%. However, GBS screening in pregnant women and antibiotic prophylaxis during labor has significantly reduced the rate of morbidity and mortality in newborns associated with GBS [1].

GBS is currently divided into 10 serotypes based on specific capsular polysaccharides that constitute a virulence factor, through which GBS eludes the host immune response. This group includes nine historically known serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII) and a further (IX) of more recent identification [2]. Although some research has been carried out on GBS in Vietnam, there is still little scientific understanding of GBS serotypes. According to a study at Nghe An obstetrics and pediatrics hospital in 2019, serotype III has the highest proportion, followed by serotypes V, Ia, and VI [3]. Determining serotypes not only contributes to identify the molecular epidemiological characteristics of GBS but also lays the foundation for research in vaccines to prevent GBS infection in pregnant women. Therefore, in the present study, we proposed to fill this gap, by providing an overview of GBS serotype III in Hanoi.

2. MATERIALS AND METHODS

2.1. Materials

- Research subjects: Women who are 35-37 weeks pregnant.

*Corresponding author
Email: dranhk61@gmail.com **Phone:** (+84) 989255773 **Https:**//doi.org/10.52163/yhc.v65i13.1802

- Inclusion criteria: Pregnant women who are at a gestational age of 35-37 weeks and agree to participate in the study.

- Exclusion criteria: Pregnant women used antibiotics or vaginal suppositories within 7 days before sample collection were removed from the study.

- Location and time: This study was conducted at the Military Hospital 103 (MH103), Ha Dong General Hospital (HDGH), Hanoi Obstetrics & Gynecology Hospital (HOGH) from October 2021 to May 2022.

2.2. Methods

- Research design: This was a cross-sectional study.

- Data collection:

Sample size: The sample size was calculated by using a single proportion formula with margin of ε = 0.15, confidence interval = 95%, and prevalence of GBS infection from a previous study conducted by Nguyen Thi Lan (p = 0.174%)

$$n = Z^2_{1-\alpha/2} \frac{p(1-p)}{(\varepsilon p)^2}$$

In this study, we calculated that the minimum number of participants was 800. To increase the accuracy, the study was conducted on 876 pregnant women.

- Research process:

Specimen collection, handling, and transport: A total of 876 vaginal-rectal samples were collected from pregnant women at Military Hospital 103, Ha Dong General Hospital, and Hanoi Obstetrics & Gynecology Hospital from October 2021 to May 2022. Vaginal-rectal swabs were collected by using a single flocked swab before vaginal examinations. First, the swab was inserted about 2 cm into the vagina without the use of a speculum, and then the same swab was inserted approximately 2.5 cm through the anal sphincter. The samples collected at MH103 and HDGH were immediately transferred to the Microbiology Department of MH103, and those gathered at HOGH were transported to the Microbiology Department of HOGH within two hours after collection. Socio-demographic and clinical data were collected by using a structured questionnaire.

GBS identification: GBS was identified following the protocol from the American Society for Microbiology[4]. To summarize, the swabs were placed in Todd-Hewitt broth plus antibiotics (Nalidixic Acid 15mg/L, Colistin sulfate 10mg/L, Melab Diagnostics, Vietnam) and incubated aerobically at 35 - 37°C for 18 - 24 hours. Subsequently, 10 µl of each broth was subcultured on Columbia agar plates with 5% sheep blood (Oxoid, UK) and Chromogenic Strepto B (Melab Diagnostics, Vietnam). The plates were then incubated for 18 - 24 hours at 35 - 37°C in 5% CO_2. If a GBS colony was not detected, the plates were re-incubated and examined after 48 hours. All suspected GBS colonies that appeared beta-haemolytic or nonhemolytic, and were Gram-positive cocci and catalase-negative cocci, were performed the CAMP (Christie - Atkins - Munch - Peterson) test and/or Lancefield grouping by latex agglutination test (Mast Strep, Mast Group, UK). Controls included Streptococcus pyogenes ATCC 19615, Streptococcus agalactiae ATCC 13813, and Staphylococcus aureus ATCC 25923 (Thermo Scientific™, Singapore). Last, GBS isolates that were positive or indeterminable were further confirmed using polymerase chain reaction (PCR). The isolated GBS were then stored at -20°C for subsequent molecular examination.

Genomic DNA extraction: In this study, bacterial DNA was extracted using the QIAamp DNA Mini Kit (Cat. No 51304, QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was tested for concentration using the NanoDropTM 2000 at 260 nm (Thermo Fisher Scientific, USA). The DNA after extraction was stored at -20°C until PCR reactions were performed.

Molecular detection of GBS: GBS isolates were performed using the specific primer pairs of dltS-F (5'-AGG AAT ACC AGG CGA TGA ACC GAT-3') and dltS-R (5'TGC TCT AAT TCT CCC CTT ATG GC-3') (1st BASE, Singapore) for the dltS gene. The PCR mixture, consisting of 2 µl of template DNA, 7.6 µl of water, 10 µl of 2X PCR Master Mix (Promega Corporation, USA), and 0.4 µl of primer with a final concentration of 10 pmol, was prepared in a final volume of 20 µl. Amplification was carried out in an automated PCR machine with the following cycling parameters: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 60 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. Additionally, all reference GBS species were subjected to PCR as controls.

Molecular serotyping using multiplex-PCR: Three sets of multiplex PCR reactions for serotype Ia, Ib, II, and III; IV and V; VI, VII and VIII were carried out. Serotype IX was identified by PCR technique. The final reaction volume was 20 µl, consisting of 7.5 µl of water, 10 µl of 2X PCR Master Mix, 0.5 µl of working primers with final concentration of 10 pmol, and 2 µl template DNA. The thermal cycling conditions were as follows: 95°C for 5 minutes as initial denaturation for one cycle, 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, for 35 cycles, with a final extension at 72°C for 5 minutes. One percent agarose gel electrophoresis was performed on PCR products.

Table 1. Primers for determination of GBS serotypes

Serotype	Primer name	Sequence (5' to 3')	Gene target	Amplicon size(s) (bp)
Ia	Ia-F	GGTCAGACTGGATTAATGGTATGC	*cps1Ah*	521 and 1.826
	Ia-R	GTAGAAATAGCCTATATACGTTGAATGC		
Ib	Ib-F	TAAACGAGAATGGAATATCACAAACC	*cps1bJ*	770
	Ib-R	GAATTAACTTCAATCCCTAAACAATATCG	*cpsIbK*	
II	II-F	GCTTCAGTAAGTATTGTAAGACGATAG	*cps2K*	397
	II-R	TTCTCTAGGAAATCAAATAATTCTATAGGG		
III	III-F	TCCGTACTACAACAGACTCATCC	*cps1a/2/3I*	1.826
	III-R	AGTAACCGTCCATACATTCTATAAGC		
IV	IV-F	GGTGGTAATCCTAAGAGTGAACTGT	*cps4N*	578
	IV-R	CCTCCCCAATTTCGTCCATAATGGT		
V	V-F	GAGGCCAATCAGTTGCACGTAA	*cps5O*	701
	V-R	AACCTTCTCCTTCACACTAATCCT		
VI	VI-F	GGACTTGAGATGGCAGAAGGTGAA	*cps6I*	487
	VI-R	CTGTCGGACTATCCTGATGAATCTC		
VII	VII-F	CCTGGAGAGAACAATGTCCAGAT	*cps7M*	371
	VII-R	GCTGGTCGTGATTTCTACACA		
VIII	VIII-F	AGGTCAACCACTATATAGCGA	*cps8J*	282
	VIII-R	TCTTCAAATTCCGCTGACTT		
IX	VII-IX-F	CTGTAATTGGAGGAATGTGGATCG	*cpsI*	229
	IX-R	AATCATCTTCATAATTTATCTCCCATT		

- Analytical statistics: Statistical analysis was performed using SPSS 20.0 software. The Chi-square or Fisher's exact test was used to compare categorical variables. Fisher's exact test was used for variables with less than five in at least one cell. A p-value <0.05 was considered significant.

2.3. Ethics

The study was conducted under the approval of the Ethics Committee for Biomedical Research of the MH103 under Decision No.08/CNCTh-HĐĐĐ dated January 06, 2023. Information relating to the research is kept strictly confidential and used only for scientific purposes. The research team is committed to complying with ethical principles and having no conflicts of interest in the study.

3. RESULTS

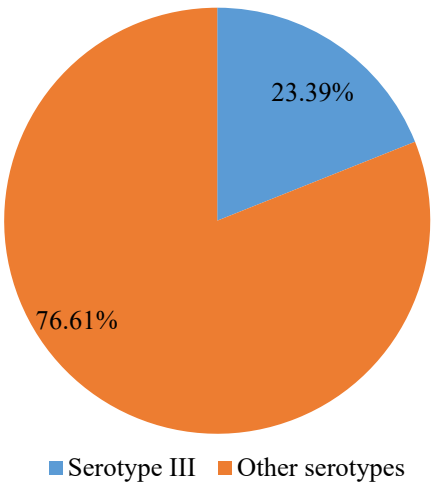

Figure 1. Prevalence of Group B Streptococcus Serotype III among GBS isolates

Out of 171 GBS isolated from pregnant women, the prevalence of serotype III was the highest with 40 isolates (23.39%). Serotypes Ia, Ib, II, V, VI, and VII accounted for the remaining 76.61%. No serotype IV, XIII or XI was identified.

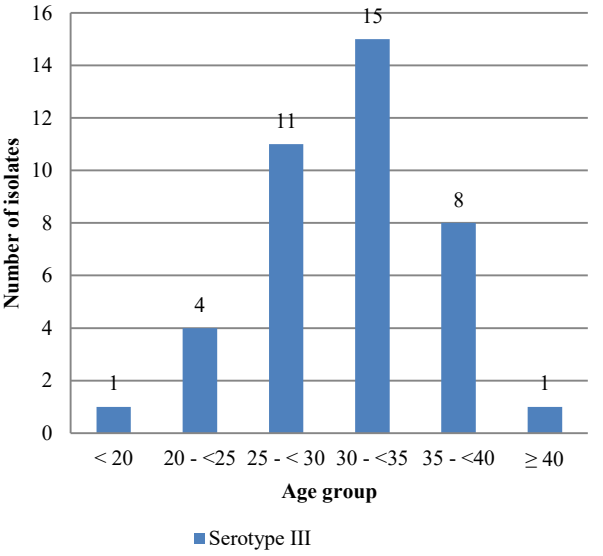

Figure 2. Distribution of age for serotype III

Figure 2 illustrates the distribution of serotype III among different age groups. The highest number of serotype III infections occurred in the 30 to under 35-year-old age group, accounting for 15 isolates of serotype III cases. The 25 to under 30-year-old group followed with 11 isolates, and the 35 to under 40-year-old group with 8 isolates. In contrast, both the youngest (<20 years old) and oldest (≥40 years old) age groups had only 1 isolate each.

Table 2. Distribution of pregnancy history for serotype III

Pregnancy history		Serotype III		Other serotypes		p
		n	%	n	%	
Miscarriage, stillbirth	Yes	6	15.0	24	18.32	p = 0.63
	No	34	85.0	107	81.68	
Preterm birth	Yes	0	0	6	4.6	p = 0.34
	No	40	100	125	95.4	

The prevalence of serotype III GBS was 15.0% among women with a history of miscarriage or stillbirth and 0% among those with a history of preterm birth. However, these differences were not statistically significant (p=0.63 for miscarriage/stillbirth; p=0.17 for preterm birth).

Table 3. Distribution of genitourinary tract infections (GTIs) history for serotype III

Symptoms of genitourinary tract infection		Serotype III		Other serotypes		p
		n	%	n	%	
Genitourinary tract infections	Yes	13	32.5	37	28.24	p = 0.6
	No	27	67.5	94	71.76	
Number of genitourinary tract infections	1 time	11	84.62	19	51.35	p = 0.04
	≥ 2 times	2	15.38	18	48.65	
Genital itching	Yes	8	20	15	11.45	p = 0.17
	No	32	80	116	88.55	

Table 3 illustrates the distribution of genitourinary tract infections history among pregnant women with GBS serotype III compared to those with other serotypes. There was a slightly higher prevalence of GTIs between pregnant women infected with serotype III (32.5%) and other serotypes (28.24%). A significant finding was that individuals with GBS serotype III are more likely to experience a single occurrence of GTIs (84.62%) than multiple occurrences (15.38%). In contrast, pregnant women with other serotypes have a higher rate of recurrent infections (48.65% experiencing ≥ 2 times). Genital itching was higher in pregnant women with GBS serotype III infection (20%) than those with other serotypes (11.45%).

4. DISCUSSION

During the study period from October 2021 to May 2022, we isolated 171 GBS strains, of which 40 were serotype III with the highest prevalence at 23.39%. This result was similar to the study reported by Nguyen Quang Hanh (2019) at Nghe An Obstetrics and Pediatrics Hospital, where serotype III also dominated among GBS isolates. However, in Nguyen Quang Hanh's study, the prevalence of serotype III was 39.1%, higher than our study results [3]. Research by Yifei Chen (2023) in China showed that serotype III accounted for 43.09% of eight serotypes [5]. According to research by Carlo Genovese (2020) in Italy, serotype III also dominated with a prevalence of 34.9% among isolated GBS in pregnant women at 37-39 weeks of gestation [6]. Interestingly, in some regions, the prevalence of serotype III has been reported to be relatively low. In a study by A. Botelho in Brazil (2008-2015), serotype III had a rate of only 6.8%, ranking sixth among the seven serotype isolates [7]. A meta-analysis by M. Gizachew (2019) across 21 African countries found that the prevalence of serotype III was 19.7%, ranking second after serotype V (29.2%) [8]. These variations in serotype distribution across studies could be attributed to several factors, including geographic location, population demographics, sampling methods, sampling time, and technical testing differences. However, the proportion of GBS-isolated strains were not typable (NT) likely due to the technical limitations of the serotyping methods.

It is well-established that serotype III is the most virulent among GBS strains, and is often associated with severe infections, including meningoencephalitis. Therefore, the data on the prevalence of serotype III in our study provide significant insights into the epidemiology of GBS at 35-37 weeks pregnant women in Hanoi. The dominance of this serotype in the pregnant population underscores the necessity for targeted preventive measures, such as the development of vaccines specifically against serotype III. In addition to preventive strategies, our findings suggest the need for continued surveillance to monitor changes in the prevalence and distribution of GBS serotypes. Surveillance data can be critical for adjusting health policies and clinical practices to better protect mothers and infants from GBS-related complications. Longitudinal studies are needed to explore the correlations between GBS serotypes and pathogenicity over time and across different populations.

Our study showed that women in their early 30s were more likely to be infected with GBS serotype III during pregnancy. The younger women (<20 years) and older women (≥40 years) had only one case of GBS serotype III infection. However, the small sample sizes for these age brackets limited the ability to draw definitive conclusions. Studies from various countries have shown similar age-related trends, with the peak prevalence typically occurring in women aged 25 to 35. This may be due to higher reproductive activity and greater awareness of antenatal care visits in this age group, which may increase the likelihood of GBS detection. Our results indicated that there was no significant association between obstetric history (miscarriage or preterm birth) and the prevalence of serotype III GBS colonization in pregnant women. The findings of this study provided insight into the distribution and characteristics of genitourinary tract infections associated with serotype III compared to other serotypes. One of the most notable findings in this study was the lower recurrence rate of GTIs in patients with serotype III. Only 15.38% of patients with serotype III experienced multiple infections, compared to 48.65% of patients with other serotypes, and this difference was significantly significant (p = 0.04). During pregnancy the vagina dilates, the vaginal mucosa increases the folds and papillae. Under the influence of progesterone, the vaginal mucosa sheds a lot and combined with vaginal fluid, it will create favorable conditions for many pathogenic bacteria in general and GBS in particular [1]. The difference between serotype III and others in GTIs is possibly due to differences in virulence factors that affect colonization and persistence of GBS.

The limitation of this study is that it was conducted in only three hospitals in Hanoi, which may not reflect the GBS serotype III distribution in other parts of Vietnam. In the future, studies of large sample sizes in Vietnam are required. Moreover, a longitudinal design might offer insights into how GBS colonization changes over time and impacts on pregnancy and neonatal outcomes.

5. CONCLUSION

This study set out to gain a better understanding of GBS serotype III. This study has identified that the prevalence of serotype III was the highest with 23.39% (40 strains) among 171 positive GBS isolates. The research has also shown that pregnant women aged from 30 to 35 are dominant with 15 cases. Serotype III made no significant difference to others in terms of pregnancy

history and GTIs. A key policy priority should be to plan for the long-term care of pregnant women in GBS screening and intrapartum antimicrobial prophylaxis.

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