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Original article

CHROMagar™ Strep B for detecting group B *Streptococcus* in pregnant women at 35th to 37th of gestation

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Abstract: ***Introduction:*** Group B *Streptococcus* (GBS) is one of the common causes of neonatal sepsis spreading from mothers to newborns. A common method to isolate and identify GBS is using Blood agar which delivers results in at least 48 hours. Although chromogenic culture media including CHROMagar™ StrepB, can develop colored colonies for detecting pathogenic bacteria easily, there has not been approached GBS isolation in Vietnam. This study was conducted to find out the GBS infection ratio in pregnant women at the 35th – 37th week of gestation. Furthermore, this research evaluates the efficacy of CHROMagar™ StrepB media and Blood agar in GBS detection as well. ***Method:*** In a cross-sectional survey, a total of 258 pregnant women at 35th to 37th of gestation screened for GBS at Thuan Kieu General Clinic from 04/2021 to 12/2021 were recruited. Rectovaginal swabs from these patients were cultured on Blood agar and CHROMagar™ StrepB. We performed data analysis using SPSS ver 20, p<0.05 was statistically significant. ***Result:*** Overall, out of 258 participants, 52 (20.16%) were GBS carriers. CHROMagar™ StrepB has significantly higher sensitivity than blood agar if spending a similar time (1.52 fold, p-value < 0.001), or event training a shortened time (18 hours and 48 hours), CHROMagar™ StrepB media is still more sensitive than blood agar (1.16 fold, p-value 0.044). ***Conclusion:*** In this study, the GBS infection ratio in pregnant women at 35-37 weeks of gestation at Thuan Kieu General Clinic is 20.16%. Culturing vaginal-rectal specimens on CHROMagar™ StrepB medium is higher sensitivity and rapidly than blood agar for GBS detection. CHROMagar™ StrepB should be used to get more effective in identifying GBS carriers in near-term pregnant women.

Keywords: cross-sectional study; GBS; Group B streptococcus; CHROMagar™ StrepB.

1. INTRODUCTION

GBS is a Gram-positive coccus that lives in women's gastrointestinal and genital tract and doesn't cause any symptoms in most situations. However, by spreading from mothers to newborns when they go into labor or pregnant's amniotic membrane breaks, GBS can cause neonatal infections such as hypothermia, meningitis, and sepsis, which is the main cause of perinatal death [1]. Therefore, GBS in the vagina should be screened to detect GBS colonization in pregnant women.

In 1996, the Centers for Disease Control and Prevention (CDC) recommended screening for GBS with the cooperation of many professional organizations. According to CDC guidelines in 2010, GBS screening is recommended for all pregnant women at 35-37 weeks of gestation. Acceptable media are Tryptic Soy Agar with 5% sheep blood or Columbia

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Agar with 5% sheep blood (blood agar), Columbia Agar with colistin and nalidixic acid, and Chromogenic Agar [2]. Chromogenic Agar has been developed yielding GBS colonies with a unique predictive color and evaluated for genital specimens [3]. In the case of using CHROMagar™ StrepB media, GBS colonies are lilac purple whereas others are blue, pink, or white colonies.

There has been much research about using Chromogenic Agar in isolation and identification of GBS in pregnant women all over the world. Many studies have shown that chromogenic Agar has a good alternative for the detection and identification of GBS, in particular, the research by Vijaya Doddaiah in India, Toyohisa Morita in Japan, Manuel Rosa-Fraile and Barbara Spellerberg in America [4, 5, 6].

However, using Blood agar is the common method for GBS isolating and identification in Vietnam. To our knowledge, there has been no research on the effects of chromogenic media in GBS screening in pregnant women in Vietnam. Therefore, this study aims to figure out the GBS infection ratio in pregnant women at 35^{th} -37^{th} weeks of gestation by using CHROMagar™ StrepB and blood agar. In addition, we also compare the efficacy of CHROMagar™ StrepB and blood agar in GBS detection.

2. MATERIALS AND METHOD

2.1. Study design and participants

A cross-sectional study was conducted at Thuan Kieu General Clinic in Ho Chi Minh City, Vietnam, from April to December 2021. The eligible participants of our study were pregnant women at 35-37 weeks of gestation who were older than 18 years old and had no mental problems. The pregnant women who had used vaginal suppositories before screening or could not define weeks of gestation were excluded from the list.

The sample size was calculated according to the one proportion-estimation formula:

$$n \geq \frac{Z^2_{1-\frac{\alpha}{2}} p(1-p)}{d^2}$$

with p = 12.6% (according to Wei Dai's study) so the sample size is equal to or more than 169 [7]. So 258 was chosen as the sample size.

2.2. Ethical clearance

Before being recruited into the study, subjects have clearly explained the benefits, purposes, and disadvantages of participating in the study, and had the right to opt out of the study. The proposal research was reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City (approval number: 34/HĐĐĐ-ĐHYD).

2.3. Data collection

After pregnant women were assessed for initial eligibility and invited to participate in our study, participants who were excluded or who withdrew from the study were noted, there were 258 eligible participants. Swabbing both the vagina introitus and the rectum is called a vaginorectal sample. A vaginorectal sample swab was collected from each eligible participant and carried to the microbiology laboratory for 4 hours. If transportation processing was delayed, the swab should be placed in a transportation medium (Stuart Amies). Each sample was an ID code, so the information of the participants was secured. Also, bias errors were avoided in collecting data. Each swab in the sample was incubated at 37ºC in selective enrichment broth (BHI with 8 µg/ml of Gentamycin) for 6 hours. Then it was subcultured onto both culture media: blood agar and CHROMagar™ StrepB agar. Blood agar plates were incubated at 37ºC for 24 hours with 5-10% CO_2, while CHROMagar™ StrepB agar plates were incubated at 37ºC for only 18 hours. β-hemolysis colonies on blood agar and lilac purple colonies on CHROMagar™ StrepB agar were identified by catalase test and CAMP test [2,3]. If a lilac purple or any suspected colony shows a negative CAMP test, a PCR test would be performed to confirm [8].

On blood agar, a sample is called true positive when it has a positive CAMP test-β-hemolysis colonies or positive PCR test. There are not any false-positive after 48 hours (24 hours incubating on blood agar, 24 hours on CAMP test) because only positive CAMP test β-hemolysis colonies can be identified as GBS. A sample is called false negative when it has no β-hemolysis colonies on blood agar but it can be detected on CHROMagar™ StrepB medium or has a positive with PCR test, and the rest of the above is true negative samples.

On CHROMagar™ StrepB medium, a sample is called true positive when it has any lilac purple colony with a positive CAMP test or GBS PCR test. A sample is called a false positive when it has any lilac purple colony with a negative CAMP test and PCR test. A sample is called false negative when it has another color colony (gradient from blue to violet) that has a positive result with the CAMP test or PCR test, and the rest of the above is true negative samples.

A pregnant woman has been identified as a GBS carrier if their sample has determined a true positive GBS colony on blood agar or CHROMagar™ StrepB medium or both media, and included positive GBS PCR. A GBS PCR test is performed if there was any suspected result on CHROMagar™ StrepB medium and blood agar.

2.4. Data analysis

Data were analyzed by using the Student Version of Statistical Package for Social Sciences (SPSS) version 20.0. Categorical variables were presented by frequency and percentages. The Paired Samples T-Tests were used to determine the differences in the effection of isolation and identification of GBS by CHROMagar™ StrepB and Blood agar. The $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. The GBS infection ratio of pregnant women

Between May 2021 and July 2021, 261 pregnant women were assessed for initial eligibility and invited to participate. The figure showed the flow of participants throughout the study. Participants who were excluded (and the reason for this) are noted, there are 258 eligible participants.

In table 1: Of the 258 participants who completed the study, we found that the average age of the participants was 30.6 ± 4.0 years old (ranging from 21 to 42 years old). There were no significant differences between the 35th-36th weeks of gestation group and the 36th-37th weeks of gestation group.

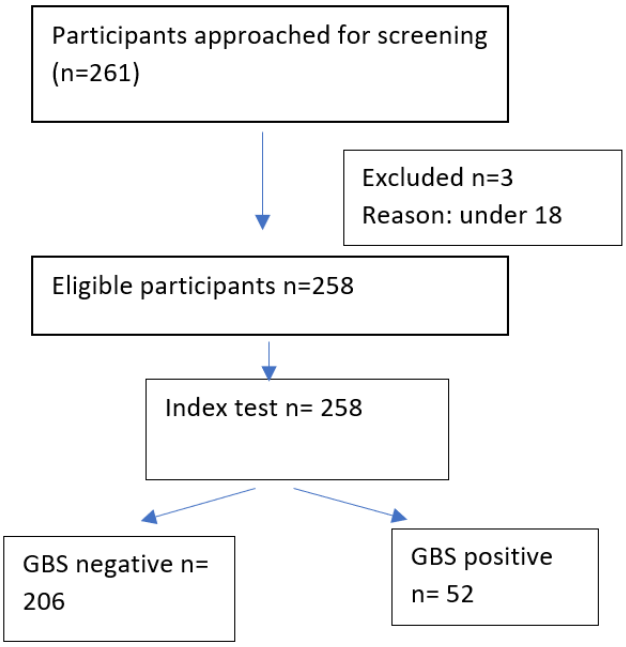

Figure 1. Flow diagram for participants in study

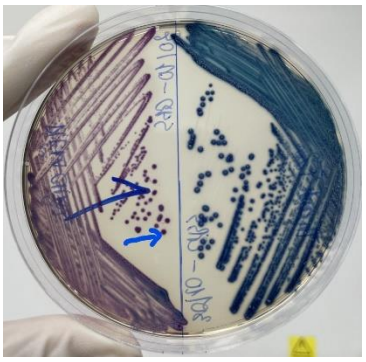

Figure 2. GBS colonies on CHROMagar™ StrepB

Figure 3. Hemolysis GBS colonies on BA

Table 1. Age and weeks of gestation

Subject's information	n	%
1. Age group distribution		
< 20	0	0.00
20 - < 25	29	11.24
25 - < 30	94	36.43
30 - < 35	86	33.33
≥ 35	49	19.00
Total, X ± SD = 30,6 ± 4.0	258	100.00
2. Weeks of gestation distribution		
35 weeks - < 36 weeks	135	52.33
36 weeks – 37 weeks	123	47.67
Total	258	100,00

In table 2: Of the 258 participants, 52 participants (20.16%) were identified as carrying GBS, accounting for 20.16% total sample.

Table 2. Results of screening GBS for pregnant women at 35-37 weeks of gestation at Thuan Kieu General Clinic

	GBS (+)	GBS (-)	Total
n	52	207	258
%	20.16	79.84	100

Figure 2: Shows the lilac color of GBS colonies on CHROMagar™ StrepB.

Figure 3: The beta-hemolysis GBS colonies on blood agar

Figure 4: The positive CAMP test on blood agar.

Figure 4. Positive CAMP test on Blood agar

3.2. The efficacy of CHROMagar™ StrepB and Blood agar in GBS detecting

Table 3: to compare the GBS detecting ability of two culture media, this table shows only positive cases detected on CHROMagar™ StrepB with lilac colony after day 1 (18 hours without CAMP test), after day 2 (42 hours with CAMP test), and on blood agar with β-hemolysis colonies and positive CAMP test after day 2 (48 hours). There was a significant difference in the GBS detecting cases between the two media. On blood agar after day 2, the GBS detecting cases was 31 while the GBS detecting case on CHROMagar™ StrepB media after day 1 was 36, and after day 2 was 47 (p-value = 0,01).

In table 4:

Case on blood agar: True-positivity: 31; False-positivity: 0 because 48 hours incubating bacteria consist of 24h CAMP test, and only positive CAMP tested β-hemolysis colonies could be identified GBS; False-negativity: 21; True-negativity: 206.

Case on CHROMagar™ StrepB after day 1: True-positivity: 36; False-positivity: 0; False-negativity: 16; True-negativity: 206.

Case on CHROMagar™ StrepB after day 2: True-positivity: 47; False-positivity: 0; False-negativity: 5; True-negativity: 206.

So when compared with blood agar, CHROMagar™ StrepB was significantly more sensitive: After day 1, the sensitivity was increased 1.16 fold (p-value=0.024) even spending less time than blood agar (18h and 48h), and after day 2, the sensitivity was seriously increased 1.52 fold (p-value < 0.001).

Table 3. Compare GBS sreening results between two media CHROMagar™ StrepB (CHROM-B) and blood agar (BA)

Media		GBS (+)		p - value
	Day	n	%	
CHROM - B	D1 (18h)	36	13.95	0.298
	D2 (42h)	47	18.22	0.001
BA	D2 (48h)	31	12.02	

Table 4. Sensitivity and specificity of two methods CHROMagar™ StrepB (CHROM-B) and blood agar (BA)

Media	Day	True - positive	False-positive	True-negative	False-negative	Sensitivity (%)	Specificity (%)	p- value
CHROM-B	D1 (18h)	36	0	206	16	69.23	100.00	**0.024**
	D2 (42h)	47	0	206	5	90.38	100.00	< **0.001**
BA	D2 (48h)	31	0	206	21	59.62	100.00	

4. DISCUSSION

According to statistics from WHO in 2017, there was an average of 18% of pregnant women worldwide carrying GBS, ranging from 11% to 35% depending on different regions. GBS infection rate is higher in Africa and lowers in Asia [9]. Compared with the GBS infection ratio worldwide, our study has a corresponded result (20.16%).

Compared with other studies about the GBS infection ratio among pregnant women in Vietnam, instead of using vaginal swabs only, we collected the samples from both the vagina and the rectum for increasing GBS recovery. So our study shows GBS detection ratio is significantly higher than Tran Quang Hanh's study (9.20%) at Nghe An Obstetrics and Pediatrics Hospital [10] and Phung Thi Ly's study (17.5%)[11].

Compared with other studies worldwide, Kathryn Braye's study in Australia from 2006 to 2016 has a similar result as our result (21.5%) [12]. In Latin America, the study of Freitas shows that the GBS infection rate in Brazil is 24% [13]. In Africa, the study by Lucia M Lekala shows that the GBS infection rate is high (48.2%) in South Africa [14]. This is reasonable to the sanitary and economic conditions of this region. In Asia, Li – Chen Hung's study in Taiwan has results similar to our study (19.58%) [15].

Compared with all before studies, Our study used both blood agar and CHROMagar™ StrepB to identify GBS so there had fewer false-negativity results than using only one culture medium. And we found that the CHROMagar™ StrepB medium needs a shorter time to identify GBS. Besides 6 hours incubating samples in BHI with antibiotics, while GBS detecting needs at least 24 hours to isolate in blood agar and 24 hours to identify GBS with the CAMP test before, now it takes only 18 hours to isolate and identify GBS when using CHROMagar™ StrepB. At the 35th to 37th week of gestation, the labor can still happen at any time, so the GBS results should be delivered to pregnant women early. Even sometimes purple-like colonies' appearance can take 18-24 hours to do the CAMP test, CHROMagar™ StrepB medium still shortens the time needed to give results.

In this study, we could see CHROMagar™ StrepB has significantly higher sensitivity than blood agar if spending a similar time(1.52 fold, p-value < 0.001), or even taking a shortened time (18 hours and 48 hours), CHROMagar™ StrepB media is still more sensitive than blood agar (1.16 fold, p-value 0,044). This result is similar to studies of Vu Thi Kim Lien and Fernanda de-Paris sensitivities of Blood agar are 45.45% and 59.15% [16, 17]. The sensitivity of CHROMagar™ StrepB is also high in the study of Vijaya Doddaiah và Gaurav Kwatra [3, 18]. In general, Chromogenic Agar has higher sensitivity than Blood agar. This is shown in studies of Toyohisa Morita in Japan, Salem Nahim and

Anderson J. Anderson in Australia, and Poisson Didier-Marc in France [5, 19, 20].

There were 16 false-negative samples on Blood agar in this study while they could be recognized as lilac colonies in CHROMagar™ StrepB media: consisting of 2 samples were covered by rectal flora,13 samples with very weak hemolysis colonies, and 1 sample with non-hemolysis colony. Because CHROMagar™ StrepB media can select against Gram-negative bacilli-bacteria, and normal flora [19]. Moreover, CHROMagar™ StrepB can also detect non-hemolytic GBS. GBS population consists of 1-3% non-hemolytic GBS. Mutations in synthesis or transporter that normally export the β-hemolysin extracellularly can create non-hemolytic GBS [21].

There were 11 samples with purple-like colonies, and they were detected after 24 hours of the CAMP test, CAMP test is suggested to do if there have any purple-like colonies in CHROMagar™ StrepB media.

There were 5 samples with positive PCR tests but couldn't be detected either CHROMagar™ StrepB media or blood agar. These colonies are β-hemolysis on blood agar and purple-like on CHROMagar™ StrepB media with the negative CAMP test.

Besides those advantages, CHROMagar™ StrepB media is more expensive than Blood agar. This is also a factor that laboratories need to consider. In this study, both Blood agar and CHROMagar™ StrepB were used to identify GBS so there had fewer false-negativity results than using only one culture media. However, this study also had limitations. First, our study was small (n = 258 and positive GBS = 52) and was not multicenter research, so the denominator is not highly representative. Second, we did not observe the GBS colonies on CHROMagar™ StrepB medium on the timeline (ex: after 12h, 18h, 24h…). So we recommend an accurate time for isolating GBS with CHROMagar™ StrepB should be done in the next studies.

Conclusion

In conclusion, the GBS infection ratio in pregnant women at 35-37 weeks of gestation at Thuan Kieu General Clinic is 20.16%. CHROMagar™ StrepB is suggested using because of its higher sensitivity and saving time for results. But the CAMP test or PCR test should be done for light purple, violet colonies, and other GBS suspected colonies on CHROMagar™ StrepB. The research needs to be expended at another medical center in Ho Chi Minh City and in Vietnam, and the optimum time for GBS isolating with CHROMagar™ StrepB should be mentioned in the next studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

LTMN and STN performed the study's experiments, collected the data, and wrote the report. NBT and ATTN participated in the development of the study design, the analysis and interpretation of the data, and the writing of the report. All authors read and approved the manuscript.

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