

RESEARCH ARTICLE

Group B streptococcus detection in China: comparison of different screening methods and different sampling sites

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ABSTRACT

Objectives: To evaluate the use of real-time polymerase chain reaction (RT-PCR) and bacterial culture methods to detect group B streptococcus (GBS) in Chinese pregnant women in the third trimester; to separately assess the prevalence of rectal and vaginal GBS colonization; and to determine the antimicrobial susceptibility pattern of the isolates.

Methodology: Samples were collected from 505 women at 35 and 37 weeks gestation at the Peking Union Medical College Hospital. Bacterial culture and RT-PCR were performed. Antimicrobial susceptibility to commonly used antibiotics was also analyzed.

Results: The overall GBS colonization rate was 7.5%. The colonization rate, sensitivity, and negative predictive value of the bacterial culture method were 2.8%, 36.8%, and 95.1%, respectively, and these values were 7.3%, 97.4%, and 99.8%, respectively, for PCR ($p < 0.001$). The GBS colonization rate of the rectum (6.7%) was higher than that of the vagina (2.8%) ($p = 0.005$). Antimicrobial susceptibility testing showed that 100% were sensitive to penicillin, cephalosporin and vancomycin.

Conclusions: RT-PCR was found to be a rapid and sensitive test for the detection of GBS colonization in Chinese pregnant women. Rectal swabbing was also important for detecting GBS colonization. β -lactams are the first-line antibiotics used for the treatment of GBS. *J Microbiol Infect Dis* 2016;6(4): 179-183

Key words: Group B streptococcus; Real-time polymerase chain reaction; Sampling sites

INTRODUCTION

Streptococcus agalactiae (group B streptococcus, GBS) is a significant cause of neonatal sepsis, pneumonia, meningitis and other serious infections [1-3]. The rectal and/or vaginal GBS colonization varies between countries ranging from 6-21.3% [4-6]. GBS is transmitted vertically to neonate through maternal amniotic fluid or birth canal during labor, causing early onset GBS infection, with an incidence of 1 to 2 per 1,000 live births [7,8].

The Centers for Disease Control and Prevention (CDC) currently recommends the screening of all pregnant women at 35-37 weeks gestation with rectovaginal cultures and intrapartum antibiotic prophylaxis (IAP) for women with GBS bacteriuria at the time of labor onset or prelabor rupture of membranes [3]. Antepartum PCR is also allowed to be performed by CDC guidelines [3]. GBS bacterial culture requires at least 48 h for results, which hin-

ders its use for intrapartum screening [9]. However, in some urgent situation, such as preterm labor or lack prenatal care, fast results can play a vital role in the timely treatment of neonates. Currently, DNA-based methods, such as polymerase chain reaction (PCR) assays offer an attractive approach for rapid detection of GBS. In PCR, the sample preparation method used and amplification target are determinants of assay performance. The *cfb* gene is a good target for GBS amplification. It is present in every GBS isolate. Further, it is a housekeeping gene with comparatively low mutation rate [10]. The anatomic sampling site is also important to gain high bacterial yields, because GBS colonization in the gastrointestinal tracts is the primary risk factor for vaginal colonization [11].

In developing countries, healthcare providers began to realize the role of GBS colonization during pregnancy. The number of reports on GBS is growing, but still not enough. The aims of this study

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were to compare the efficacy of PCR with that of a culture-based method and to investigate the GBS colonization of pregnant Chinese women. To determine an appropriate sampling method for use in developing countries, we also compared two sampling methods, and characterized the isolated GBS strains in terms of antibiotic resistance.

METHODS

Sample collection

This study was approved by the research ethics committee of Peking Union Medical College Hospital, and all of the included women gave written informed consent. Between September 2013 and April 2014, 1010 vaginal samples and 1010 rectal samples were collected from 505 pregnant women at 35-37 weeks of gestation (four samples per subject). Two samples (one vaginal and one rectal) were sent to the microbiology laboratory for bacterial culture, and the other two samples were sent for molecular tests within 1 hour. To collect the rectal specimens, a swab was carefully inserted approximately 1.5-2 cm beyond the anal sphincter and then gently rotated to touch the anal crypts. The vaginal samples were collected from the lower third of the vagina by rotating the swab 360 degrees against the vaginal wall. Women with genital fistulae and those taking antibiotics were excluded from the study.

GBS Culture

The swabs were seeded on colistin nalidixic acid Columbia agar (CNA) with 5% sheep blood. The Columbia CNA agar plates were incubated at 35°C and 5%-10% CO₂ for 24-48 h. β -Hemolytic colonies with morphology consistent with GBS were subjected to CAMP (Christie, Atkins, Munch, Petersen) test. The colonies that tested positive with the CAMP test were presumptively considered GBS-positive.

Susceptibilities to ampicillin, penicillin G, and erythromycin were assessed by Kirby-Barer (K-B) disc diffusion [12].

Real-time Polymerase chain reaction (RT-PCR)

Sample preparation and DNA extraction

The swabs were soaked in Tris Ethylene Diamine Tetraacetic Acid (EDTA) (TE) buffer (100 mM Tris-HCl and 10 mM EDTA) and suspended by vortexing at high speed for 2 min. After washing 2 times, 50 μ L of TE buffer was added to the tube, which was then vortexed at high speed for 5 min. A plasmid containing the target gene from an original strain obtained from the American Type Culture Collection (ATCC)

was used as a positive control, and TE buffer was used as a negative control. The tubes containing the specimens and the positive/negative controls were centrifuged for 2-5 sec and heated at 95°C for 2 min. Then, they were placed on ice for 2-5 min for DNA extraction [13]. The flow-through was used for PCR.

RT-PCR analysis of *cfb* gene

The positive and negative controls were assessed simultaneously in all real-time reactions. The amplified DNA targets were detected using a FAM molecular beacon at the 5' end with a StratageneMx3000P real-time PCR detection system (Agilent Strata gene, USA) with the StratageneMx3000P real-time PCR detection system (Agilent Strata gene, USA), using the Group B Streptococcus Nucleic Acid Detection Kit (Triplex International Biosciences Co., Ltd, Fu Jian, China). The kit was certificated by state food and drug administration (SFDA) (YZB/China 0102-2011). The test process required approximately 2 h.

DNA Sequencing

The results of the bacterial culture and RT-PCR methods were compared for each specimen. For discrepant results, DNA sequencing was performed to confirm the initial findings. The target gene was *cfb*, and we used the primers GBS-F (5'-AACTCAACATTTAGCAAATAAG -3') and GBS-R (5'-CGTG-TATTCCAGATTTCC -3') to generate a 260 bp fragment that differed from the RT-PCR product. The amplified PCR product was sequenced by Beijing Rui Biotechnology Limited Company, and the sequence was searched in GenBank using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information, Bethesda, MD (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Statistical analysis

Samples were considered true positives if bacterial culture or sequencing yielded positive results. The sensitivity, specificity, and positive and negative predictive values (PPVs and NPVs, respectively) of the different sampling and screening methods were calculated. Pearson's χ^2 test was used to assess the data and a p value of <0.05 was considered significant. Statistical analysis was performed using SPSS® version 16.0.

RESULTS

Bacterial culture and RT-PCR were performed separately for the rectal and vaginal samples collected

from a total of 505 women. The mean maternal age was 30.9 years (SD=3.4), and the mean gestational age at delivery was 39.3 weeks (SD=1.4). Further, 56.2% were nulliparous, and 6.4% delivered before 37 weeks gestation.

Comparison of bacterial culture and RT-PCR

A total of 38 out of 505 pregnant women (7.5%) were colonized by GBS. Of these 38 women, 14 (2.8%) tested positive by bacterial culture, and 37 (7.3%) tested positive by RT-PCR ($p=0.001$) (Table 1). We compared the sensitivity, specificity, PPV, and NPV for the bacterial culture and RT-PCR methods

(Table 2). The sensitivities of the RT-PCR and bacterial culture methods were 97.4% and 36.8%, respectively ($p<0.05$), and the NPVs were 99.8% and 95.1%, respectively ($p<0.05$).

Table 1. Comparison of bacterial bacterial culture and RT-PCR results

| RT-PCR | Bacterial culture | | |
|----------|-------------------|----------|-------|
| | Positive | Negative | Total |
| Positive | 13 | 24 | 37 |
| Negative | 1 | 467 | 468 |
| Total | 14 | 491 | 505 |

Table 2. Antepartum validity of RT-PCR compared with bacterial culture

| Method | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-------------------|-----------------|-----------------|-------------|----------------|
| Bacterial culture | 14/38 (36.8) | 467/467 (100) | 100 (14/14) | 95.1 (467/491) |
| RT-PCR | 37/38 (97.4) | 467/467 (100) | 37/37 (100) | 467/468 (99.8) |
| Chi-Square | 28.9 | - | - | 18.8 |
| p value | 0 | - | - | 0 |

Comparison of vaginal and rectal sampling

GBS was detected in both the rectal and vaginal swabs from 9 patients, in only the vaginal swabs from 5 patients, and exclusively in the rectal swabs from 24 patients. The GBS detection rate for the rectal samples was 6.7%, which was significantly higher than that for the vaginal samples (2.8%) ($p=0.005$). Rectal sampling enabled the detection of 86.8% of the GBS carriers compared with the detection of 36.8% achieved by vaginal sampling only ($p=0.000$).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing showed that the sensitivity of GBS was 100% to penicillin, cephalosporin and vancomycin, 89.5% to Chloramphenicol, 68.4% to Levofloxacin, 63.2% to Clindamycin, 52.6% to Erythromycin, 31.6% to Azithromycin, 31.6% to Tetracycline.

DISCUSSION

Although the epidemiology of GBS in the developed world is well documented, few studies have been conducted in the developing world [14]. To date, there is no national policy for systematic surveillance of GBS in China. In this study, the GBS colonization rate was 2.8% for the bacterial culture method and 7.3% for RT-PCR. Another two studies of China showed a similar maternal GBS colonization rate (7.1-7.5%) [15,16], which is lower than

other developing countries (9.5-20%) [17,18]. The difference could be due to different methodology as well as populations investigated.

A good screening test should have high sensitivity and NPV. In our study, the sensitivity of RT-PCR was 97.4% and NPV was 99.8%, which is similar to values observed in studies conducted by Bourgeois-Nicolaos N [19] and Abdelazim IA [20], who reported sensitivities of 90.9% and 98.3%, NPV of 99.2% and 99.4% respectively. The NPV of screening test is very important, because the screening results will guide the clinical implementation of antibiotic. If the result is false negative, the patient will lose a good chance for the treatment. Rapid result is another satisfying parameter of a screening test. The RT-PCR method proposed in this study requires 2 h to obtain the final results. In contrast, the bacterial culture method is time-consuming, requiring at least 48 h for full GBS identification. Therefore, PCR has the advantage to screen women delivering preterm or women without prenatal care. The percentage of women without prenatal care is rather high in developing countries with large population like China.

In this study, 24 samples tested positive with RT-PCR and negative with bacterial culture. The possible reasons for these results are as follows: i) RT-PCR detects bacterial DNA and not viable bacterial colonies; ii) other microorganisms may inhibit the growth of GBS; iii) GBS non-beta-hemolytic variant may exist. Additionally, another sample tested posi-

tive by bacterial culture and negative by RT-PCR. A repeat PCR of the false-negative sample yielded a positive result, indicating that low GBS level in the specimen and high viscosity of discharge may affect PCR result.

The intestinal tract appears to be a primary reservoir for GBS and the likely source of vaginal colonization in pregnant women [21]. We found that the combination of rectal and vaginal sampling was the best method for detecting GBS colonization in pregnant women because colonization of the rectal samples (6.7%) was higher than that of the vaginal samples (2.8%). Our results are in accordance with those of previous studies [22]. In an analysis of 651 specimens, the use of both vaginal and rectal swabs detected 97.3% of total GBS carrier, while vaginal sampling alone enabled detection of 31.8% [23]. In our study, rectal sampling enabled the detection of 86.8% of the GBS carriers, compared with 36.8% of carriers detected by vaginal sampling only, further highlighting the limitation of the use of this type of sampling alone. Unfortunately, most obstetric departments in China still only use vaginal sampling to assess GBS positivity.

Our results provide some information concerning GBS isolates obtained from Chinese pregnant women, though the GBS isolates are from only one hospital. In this study, 100% of the isolates were sensitive to penicillin, cephalosporin, linezolid and vancomycin. The rate of resistance to clindamycin was 36.8% and that to erythromycin was 47.4%. These findings are consistent with those of a study performed in Taiwan, in which the rates of resistance to erythromycin and clindamycin were 44% and 39%, respectively [24]. In the developed countries, such as Swiss, New Zealand, Australian and Norway, resistance to clindamycin and erythromycin was found to be from 15% to 28% and from 9% to 30%, respectively in recent studies [25-27]. Penicillin is the first choice for prophylaxis and treatment of GBS infection, and resistance to this agent has been reported among few GBS isolates, implying that it could be used for empiric prophylaxis.

The limitation of this study is that GBS bacterial culture was performed on agar supplemented with sheep blood and not selective broth media, which is not available at most Chinese hospitals. A study using selective broth media is currently underway.

CONCLUSION

RT-PCR is a rapid, sensitive and specific test for the detection of GBS colonization in pregnant women,

especially those with PROM or preterm delivery or those who lack prenatal care. The GBS colonization rate of rectal samples is higher than that of vaginal samples. Rectal swabbing is an important sampling method to detect GBS colonization. Penicillin therapy remains an appropriate first-line antibiotic choice for intrapartum GBS chemoprophylaxis, and erythromycin and/or clindamycin resistance is high in the Chinese population.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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