Prevalence of Group B Streptococcus Colonization in Pregnant Women in Jiangsu, East China

CURRENT STATUS: POSTED

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DOI: 10.21203/rs.2.16719/v2

SUBJECT AREAS
Maternal & Fetal Medicine

KEYWORDS
Group B streptococcus, prevalence, colonization, antibiotic resistance
Abstract
Background: Group B streptococcus (GBS) is the leading cause of early-onset neonatal sepsis. However, GBS was infrequently reported in the developing world in contrast to western countries. This study assessed the prevalence of GBS colonization among pregnant women in Jiangsu, East China and revealed the difference of GBS infection between culture and PCR. Methods: A total of 16,184 pregnant women at 34 to 37 weeks, gestation aged 16-47 years were recruited from Nanjing Kingmed Diagnostics. There were 9022 pregnant women receiving GBS screening by PCR detection only. 7162 pregnant women received GBS screening by bacterial culture and GBS-positive samples were tested for antibiotic resistance. Results: The overall GBS positive rate was 8.7% by PCR and 3.5% by culture. There was no significant age difference of GBS infection, but the 25-29 age group and people aged over 40 years should pay more attention. The 249 GBS-positive samples which detected by culture were all sensitive to penicillin. The prevalence of resistance to erythromycin, clindamycin and levofloxacin was 77.5%, 68.3% and 52.2%, respectively. Conclusions: This study revealed the data on the prevalence of GBS colonization in pregnant women in Jiangsu, East China. And it compared the difference of GBS infection between culture and PCR. PCR was expected to become a quick method in pregnancy women conventional detection of GBS infection.

Backgrounds
Group B streptococcus (GBS) is the main pathogen of perinatal infection. It is not only the leading cause of early-onset neonatal sepsis and meningitis (first 28 days of life), but also has been associated with preterm labor, premature rupture of membranes, chorioamnionitis, and puerperal and fetal infections in many countries[1-3]. Screening of pregnant women for GBS colonization during the third trimester, coupled with targeted intrapartum antibiotic prophylaxis (IAP) of colonized women during labor, has reduced the incidence of invasive GBS disease in western countries[4]. GBS detection and identification has become more commonplace, due to the availability of polymerase chain reaction (PCR) technology[5]. However, the traditional method of culture of GBS is still the gold standard. Penicillin and clindamycin are the first and second line antibiotic recommendations in most countries.
Penicillin, ampicillin and cefepime are the main drugs of choice to treat GBS infection in China. Vancomycin, macrolides (such as erythromycin, azithromycin, and clarithromycin), and lincosamides (clindamycin) may be used as the alternative drugs for patients allergic to penicillin or cephalosporins[6-9].

In this study, we investigated the GBS colonization rate in pregnant women in Jiangsu, China. At the same time, we compared the difference in the detection rate of GBS between the two methods of culture and PCR and described the sensitivity of GBS to different antibiotics.

Methods

Study population

Between June of 2017 and June of 2019, the pregnant women at 34 to 37 weeks' gestation who resided in Jiangsu Province and received GBS screening at Nanjing KingMed Diagnostics were studied for participation. The pregnant women had not received antibiotic treatment for at least two weeks prior to recruitment into the sample collection[10]. We performed an analysis of 16,184 women aged 16–47 years, including 9022 pregnant women who received GBS screening by PCR and 7162 by culture. GBS-positive samples were tested for antibiotic resistance by automatic microbial identification and drug sensitivity analysis system.

Specimen collection

A set of vagino-rectal swab samples consisting of two swabs were taken. The specific operation steps were carried out according to the method recommended by the 2002 CDC. The accurately labeled swabs were placed in a cooler box containing ice packs, and transported to the laboratory at Nanjing KingMed Diagnostics within 2-4 hours of collection. Specimens were collected by an obstetrician and taken as part as standard care, over the course of two years from 16,184 pregnant women.

PCR assays

GBS DNA were detected using the Group B Streptococcus (GBS) nucleic acid detection kit (BioChain (Beijing) Science & Technology. Inc.). Firstly, each vaginal or rectal swab specimen was combined with 1ml of normal saline (0.9% NaCl). A set of vagino-rectal swab sample was consisting of 500µl mixed liquid of vaginal specimen and 500µl mixed liquid of rectal swab specimen. DNA was extracted
from the mixed liquid following the manufacturer’s instructions, then 100 ng (5µl) GBS DNA was used as template and added into 35 µl reaction mixture. PCR was done using conditions described in the manufacturer’s instructions on a ABI PCR system 7500 version 2.3 for the amplification.

Microbiology (culture)
Cotton swab samples (a set of vagino-rectal swab samples) from pregnant mothers were inoculated into Todd-Hewitt culture broth, subcultured on Columbia blood agar to which 5% sheep blood has been added (Oxoid, United Kingdom), then incubated at 37℃ in ambient air for 24-48 h. The colonies on the solid media were presumptively identified as Group B Streptococcus if they forming light red to dark red colonies on CHROMagarStrepB.

Antimicrobial susceptibility test
GBS-positive samples were tested for antibiotic resistance by VITEK 2 Compact system (France). The disk diffusion method was used to measure resistance to penicillin, ampicillin, cefepime, cefotaxime, ergomycin, clindamycin, chloramphenicol, linezolid, vancomycin and levofloxacin according to the Clinical and Laboratory Standards Institute (CLSI) standards [11].

Statistical analysis
Statistical analyses were performed using SPSS version 19.0 (IBM, Armork, NY, USA). GBS positive rate was estimated by a proportion and summarized as a percentage and proportions compared using exact binomial 95% confidence intervals (95% CI). The chi-squared ($\chi^2$) was used to compare proportions of different age groups. A $p$-value of <0.05 was considered statistically significant.

Results
The prevalence of GBS infection
A total of 16,184 pregnant women were enrolled in the study. 789 participants (8.7%, 95% CI: 8.2%-9.3%) out of 9022 women studied by PCR showed GBS colonization, while 249 (3.5%, 95% CI: 3.1%-3.9%) of 7162 women investigated by culture were colonized (Table1). The average positive rate of GBS infection is 6.4% (95% CI: 6.0%-6.8%).

Prevalence of GBS colonization among pregnant women of different age groups
The analysis of the prevalence of positive GBS results were presented by different age groups (≤24
years, 25-29 years, 30-34 years, 35-39 years and ≥40 years). There were both no obvious difference among different age groups by PCR ($P=0.161$) and by culture ($P=0.28$).

Among the women by PCR, the highest rate of GBS colonization (9.4%, 95% CI: 8.5-10.4%) was the 25-29 age group. It was significantly different from the under 24 age group ($P=0.011$), but no difference from other groups. In the women by culture, people aged over 40 years had the highest prevalence rate (7.1%, 95% CI: 2.0-12.3%). It was significantly different from the under 24 age group and 30-34 age group, but it was no difference from other age groups (Table 2).

**Antimicrobial susceptibility**

Antimicrobial susceptibility test for GBS colonized samples in the women by culture, all samples were susceptible to penicillin, linezolid and vancomycin. The prevalence of resistance to erythromycin, clindamycin and levofloxacin was 77.5%, 68.3% and 52.2%, respectively (Table 3).

**Discussion**

This study showed the prevalence of GBS colonization in pregnant women in Jiangsu, East China. The pregnant women among 25-29 years old and aged over 40 years should pay more attention in this area. And we compared the difference of GBS infection between culture and PCR. PCR was expected to become a quick method in pregnancy women conventional detection of GBS infection. The GBS-positive samples which detected by culture were all sensitive to penicillin.

GBS infection can be transient or persistent during pregnancy, which inevitably leads to different results of GBS in the same pregnant woman at different times of pregnancy[1,12]. Therefore, we should choose the same stage of pregnant women when studying the infection rate of GBS. There are regional differences of GBS colonization in pregnant women. For example, the reported prevalence of GBS for Africa is 22.4%, Southeast Asia is 11.1% and Taiwan is 23.7%[13-14]. Unfortunately, large-scale multicenter epidemiological studies on maternal GBS colonization in mainland China are still rare[15].

So far, there have been many regional studies on the rate of GBS colonization in China. It was reported that the prevalence of GBS for Beijing was 7.1% and Qingdao in Shandong Province was
10.61% in Northern China\textsuperscript{[16-17]}; Shanghai was 3.7% and Nanjing was 4.16% in Eastern China\textsuperscript{[18-19]}; Chongqing was 7.05% and Chengdu in Sichuan Province was 5.02% in Southern China\textsuperscript{[20-21]}. The infection rates of GBS vary widely in different parts of China, and the prevalence of GBS in northern region is significantly higher than the eastern region. In our study, the rate of GBS colonization obtained by culture was 3.5% and by PCR was 8.7%, in Jiangsu, China. The average positive rate of GBS infection was 6.4%. The rate in our study was lower than the northern region. The main reason for this difference may be related to local economic levels and environmental factors. Another important factor is the neglect of detection method of GBS.

In our study, the rate of GBS colonization obtained by culture only (3.5%) was much lower than the rate obtained by PCR (8.7%) in Jiangsu, China. This is mainly because PCR is a rapid method which more sensitive and specific than culture. It may be due to the presence of nonviable GBS or low bacterial load in vaginal swabs, which cannot be detected by culture, but their DNA could be present for PCR amplification\textsuperscript{[22-23]}. Some pregnant women colonized by GBS might be missed only using a culture method.

Among the different age groups, the 25-29 age group and people aged over 40 years should pay more attention. It may be related with the sexually active life, history of induced abortion and higher estrogen levels during pregnancy in these age groups. These factors can cause micro-environmental changes in the genital tract bacteria. This phenomenon will be continue to focus on in future research.

IAP agents and dosing should be administered basing on the test results of GBS among pregnant women according to the Centers for Disease Control (CDC) guidelines. Penicillin remains the agent of choice for IAP, with ampicillin as an acceptable alternative in China. Antimicrobial susceptibility testing should be ordered for antenatal GBS cultures performed on penicillin-allergic women at high risk for anaphylaxis. Then, the sensitive antibiotic could be chosen according to the results of antimicrobial susceptibility testing.

Previous studies on GBS bacteremia in adults during 2002 to 2010 in USA had shown that erythromycin and clindamycin resistance occurred in 43.6% and 39.7% of cases, respectively\textsuperscript{[24]}. And
the prevalence of resistance to erythromycin and clindamycin from Taiwan for the period 2006–2008 was 58.3% and 57.9%, respectively \(^{25}\). In our study, the prevalence of resistance to erythromycin and clindamycin was 77.5% and 68.3%, respectively. It was higher than the prior studies. The goal of our research is pregnant women, which is a special group of people. It may be the main cause of this difference.

**Conclusions**

In the present study, we presented the data on the prevalence of GBS colonization in pregnant women in Jiangsu, East China. At the same time, we compared the difference of GBS colonization between culture and PCR. Such data could guide interventions to control prevalence of GBS. IAP agents and dosing should be administered according to the test results of GBS among pregnant women.

**Abbreviations**

GBS: Group B Streptococcus; IAP: intrapartum antibiotic prophylaxis;

CI: Confidence interval

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Nanjing KingMed Diagnostics. The Ethics Committee of Nanjing KingMed Diagnostics concluded that no informed consent was required because the data are anonymized appropriately.

**Consent to publish**

Not applicable.

**Availability of data and materials**

The data and materials used during the study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

No funding was obtained for this study.
**Authors’ Contributions**

RW and HMQ carried out the sample collections, laboratory detection and drafted the manuscript. YMG drafted and revised the manuscript. FP and SHB participated in the design of the study and the statistical analysis. All authors read and approved the final manuscript.

**Acknowledgments**

We would like to thank Kingmed Diagnostics for providing the data used in this paper.

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