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Identification and characteristics of antimicrobial-resistant Group B *Streptococcus* isolated from maternal birth canals at a university-affiliated hospital, China

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Purpose of investigation: This study aimed to ascertain the colonization rate of Group B *Streptococcus* (GBS) in the birth canals of late-pregnant women, and to demonstrate the characteristics of the origins of antibiotic-resistance. *Materials and Methods*: A prospective survey of pregnant women from 2017 to 2019 at a university-affiliated hospital in China was conducted. A total of 275 pregnant women with a pregnancy between 35 and 37 weeks were included in the study. Vaginal secretions and rectal swabs were tested by GBS separation to identify cultures, and an agar doubling dilution method was performed to assess drug sensitivity. *Results*: In 275 pregnant women, 20 cases were GBS screen-positive for the vaginal swabs with a GBS colonization rate of 7.27%. In addition, 16 isolates from the rectal swabs were also GBS screen-positive. Of 20 isolates from the vaginal swabs, 90.0% were resistant to tetracycline, 70.0% were resistant to erythromycin, 40.0% were resistant to clindamycin, 40.0% were resistant to levofloxacin and 30.0% resistant to chloromycetin. All isolates were susceptible to penicillin G, vancomycin, and linezolid. *Conclusions*: The higher prevalence of GBS colonization among pregnant women indicated the critical need for screening GBS colonization in late-pregnant women in the region. The study revealed the resistant patterns of GBS colonization and inferred a possible mechanism of the GBS resistance to antimicrobial agents used as animal growth promotion substances.

Keywords

Group B *Streptococcus*; Pregnant woman; Colonization; Resistance

1. Introduction

Group B *Streptococcus* (GBS), also named as *Streptococcus agalactiae*, is a gram-positive β hemolytic Streptococcus, which is the normal flora of the urogenital tract and rectum. GBS is a major conditional pathogen that causes morbidity and mortality in newborn neonates in developing countries [1]. Widespread geographical variation in GBS colonization rates has been reported in approximately 10%–30% of pregnant women colonized with GBS worldwide [2]. In recent years, GBS has been one of the main pathogens leading to severe infection and death in neonates. Clinically, neonatal GBS infections are classified as either early-onset disease (EOD) or late-onset disease (LOD). Premature onset infection mainly occurs at $<$ 7 days of life, mainly showing pneumonia and respiratory failure. Late-onset infection occurs mainly via bloodstream infections caused by meningitis after ineffective treatment [3, 4]. Following neonatal exposure to the GBS, vertical transmissions occur from the colonized mother to infants during delivery. Bacteria can quickly penetrate into the bloodstream with the occurrence of EOD or LOD such as pneumonia, sepsis and meningitis [5].

Studies have shown that vertical transmission in mother-to-child is the main cause of neonatal GBS infection, especially early infection. Timely intervention in GBS colonization in pregnant women is an important measure towards reducing neonatal infection and mortality. Data for the detection of GBS isolates and characteristics are not widely available in laboratories in the Northeast China. This study aimed to determine the colonization rate of GBS in the birth canals of late-pregnant women, and to evaluate the status of antibiotic-resistance to acquire data that could be used to support clinical decision making in GBS screening, prevention, and treatment with implementation using appropriate antibiotics.

2. Materials and methods

A total of 275 women with a pregnancy between 35 and 37 weeks were enrolled from the department of obstetrics. Participants were recruited for vaginal trial production in a university-affiliated hospital in Jilin, China from October 2017 to March 2019. Pregnant women, who had not a premature rupture of membranes or premature puberty, and had a pregnancy of less than 35–37 weeks were also included except for pregnant women with a history of antibiotic use within the last month. All cases were confirmed and checked by ultrasonography during the last menstruation and early pregnancy. Human experimental protocols to collect clinical samples were approved by the ethical committees of Beihua University (formal ethical approval number: Protocol Number 2017-10-12). Written informed consent was obtained from all participants in the study.

Sample collection, GBS culture and strain identification were performed as previously recommended [6]. For pregnant women who met the established exclusion criteria, first,

the vulva secretion was wiped off without the use of a vaginoscopy. A sterile cotton swab was inserted into the vagina and rotated to collect vaginal secretions. Another swab was inserted into the anus and gently rotated in the anal sphincter at a depth of 2 to 3 cm to determine rectal secretions. Swabs were inserted into sterile bottles and immediately sent for microbial culture. All clinicians involved in sampling were trained in the sampling methods to meet the requirements of the guidelines. The collected specimens were placed in Granada TM-T media (BioMerieux, Granada, France), and cultured for 18–24 hours in an incubator at 35–37 °C. The growth of GBS was identified if the broth showed a color change from colorless to orange. When no color change was observed in broth, they were inoculated with blood agar plates (Oxoid, USA) and placed in an incubator at 35 °C and 5% carbon dioxide for 18–24 hours, to eliminate false negative results. GBS strains were identified by Lancefield antigen serotyping, the hemolysis and cAMP tests in addition to the VITEK-2 microbiology system (BioMerieux, France).

Susceptibility to a panel of seven antimicrobial agents including penicillin G, vancomycin, erythromycin, tetracycline, levofloxacin, clindamycin, and linezolid (Oxoid, Basingstoke, UK) was determined and elucidated by using an agar doubling dilution method similar to that of the Clinical and Laboratory Standards Institute (CLSI) criteria [7]. Besides, susceptibility to chloromycetin was confirmed with a Kleihauer-Betke (K-B) test as evaluation of the origins of resistance thereto. Control strains were *Streptococcus pneumoniae* ATCC 49619 and *S.* ATCC 25923. The data pertaining to all isolates were analyzed using WHONET 5.6 software. SPSS14.00 software was selected for data analysis. The measurement data are expressed as the mean ± standard deviation and percentage, the paired t test was used for comparison of experimental groups, and $P < 0.05$ was considered statistically significant.

3. Results

This study included 275 women with a pregnancy of 35–37 weeks. The women had an average age of 28.7 ± 3.5 years, a minimum age of 19 years and a maximum age of 43 years. All pregnant patient fertilities included 15 cases with high maternal age (more over 35 years), 18 cases were multipara and 257 cases were primipara; 130 cases had one or two abortion history, and only 14 cases underwent more than three abortion. All pregnant women were confirmed and checked by B-mode ultrasonography at the last menstruation and at early pregnancy. Preliminary demographic data for the patients are listed in Table 1: there were no statistical differences in age among patients ($P > 0.05$).

Of the 275 pregnant women, 20 cases were GBS screen-positive for the vaginal swabs with a colonization rate of 7.27%. In addition, 16 isolates from rectal swabs were also GBS screen-positive. A total of isolated GBS strains were beta-hemolytic on blood agar plates for subculture. The distributions of specimens and GBS screen-positive results are summarized in Table 1.

The antimicrobial susceptibility tests showed that of the 20 GBS strains isolated, 18 (90%) strains were resistant to tetracycline, 14 (70%) strains were resistant to erythromycin, eight (40%) strains were resistant to clindamycin and levofloxacin respectively, and six (30%) were resistant to chloromycetin. All isolates were susceptible to penicillin G, vancomycin, and linezolid.

We also observed the new-born outcomes in a long-term study: amongst the 20 pregnant women who had positive GBS cultures, three cases had premature rupture of the membranes, two cases had amniotic fluid contamination, two cases had oligohydramnios, and two cases had intrauterine fetal distress. There were 16 pregnant women who used penicillin as an antibiotic during the perinatal period. Among them, two cases involved natural delivery and 14 cases involved caesarean section. In the 16 pregnant women, 13 cases of the new-borns delivered were normal. There were two new-borns with neonatal pneumonia: one case was due to ABO neonatal hemolysis and was transferred to the neonatology department for treatment and was successfully cured. The four pregnant women, during the perinatal period, went without implementation of intrapartum antibiotic prophylaxis underwent natural childbirth, three new-borns showed symptoms of health-related conditions, and one case of neonatal pneumonia was found, and was transferred to the neonatology department for treatment, however, the patient prognosis was unknown owing to their discharge from hospitals without detailed information. Although three cases of neonatal pneumonia were diagnosed by radiological imaging, all new-borns received sputum culture, however, no pathogens were noted due to the unqualified sputum samples from the new-borns.

4. Discussion

Pregnancy vaginal colonization of GBS as a routine screening tool for prenatal diagnosis has now become an issue of public health concern worldwide [8, 9]. However, most of the areas and hospitals did not carry out the detection, resulting in a lack of epidemiological data related to GBS in pregnant women in China. Therefore, there is an urgent need to clarify the situation of GBS colonization and drug resistance in the birth canals of pregnant women.

In the present study, the colonization rate of GBS was 7.27%. Our findings agreed with the results in two reports from Chengdu and Beijing [10, 11]. Similar findings were also reported from those in Japan, Malaysian and Korea whose colonization rates of GBS are 8.2%, 7.5% and 8.0% respectively [12–14]. A previous study showed that the colonization rates of GBS for late-pregnant women in different regions and races ranged between 6% and 45% worldwide [15]. Variable colonization rates of GBS (8.87%–19.30%) among pregnant women were documented across different regions in China [16].

Generally, the total colonization rates of GBS were lower than those in the USA and other European countries [4, 17].

Table 1. Demographic characteristics and isolates of all subjects in the study.

Variables	High maternal age	Maternal women	Primitive women	Abortion history	Multiple miscarriage history (≥ 3)
	$n = 16$	$n = 18$	$n = 259$	$n = 131$	$n = 15$
Age (years)	38.4 ± 3.1	37.5 ± 3.4	28.7 ± 3.1	29.7 ± 2.8	30.6 ± 3.1*
GBS screen-positive for rectal swabs	2	5	15	10	3
GBS screen-positive for vaginal swabs	2	5	15	10	3

*$P > 0.05$ indicates no statistical significance in age among all patients.

There were three possible reasons for this: one was the problem of specimen transport. Although specimens were collected immediately after inoculation, some studies have shown that the positive rate of immediate inoculation of cotton swabs collected for GBS cultivation was lower than that of cotton swabs, but the positive rate of delayed inoculation was lower. Second, this study excluded pregnant women who had recently used antibiotics. Some pregnant women with premature rupture of the membranes and preterm labor were excluded from the study due to the immediate use of antibiotics. Premature rupture of the membranes and threatened premature labor often involves intrauterine infections. A considerable part of premature rupture of the membranes, premature delivery and premature birth were caused by subclinical infection, which was not detected clinically. Evidence showed that the positive rates of GBS in patients with premature rupture of the fetal membranes were higher than those in normal pregnancy [18]. The exclusion of pregnant women with premature rupture of the membranes may result in a reduction in the positive rate of GBS. Third, various countries and regions have reported visible differences in the infection rate of late-pregnant women. These different colonization rates might be attributed to the identification method of GBS, gestational age, ethnicity, areas and delivered times [19, 20]. Additionally, we selected Granada TM-T media to detect GBS colonization in pregnant women as reference reported by Manuel *et al.*, which improved the reporting results and reduced the direct cost of screening [21]. On the other hand, most of the specimens were taken from upper vaginal secretions in China. No distinct times were selected for pregnant women at 35–37 weeks, in which there was a degree of difference with standard guidelines as recommended by CDC [7].

In the present study, resistance to tetracycline and erythromycin was 90.0% and 70.0%, respectively. Resistance to clindamycin and levofloxacin was 40.0%, and 30.0% of GBS isolates were resistant to chloromycetin. All GBS isolates were 100% sensitive to penicillin G, vancomycin, and linezolid, indicating that their predictable use for empiric prophylaxis. The susceptibility patterns of GBS isolates found in the study are in line with those reported previously [22]. Macrolides including both erythromycin and clindamycin exhibited higher resistance to GBS. The incidence of erythromycin resistance is higher compared with Taiwan (58.3%) and clindamycin resistance is higher than that in the USA (38.4%), and other western countries, ranging from 11.5% to 32% [23, 24]. Highly resistant rates of erythromycin, clindamycin, and levofloxacin demonstrated that pregnancy vaginal colonization rates of GBS were globally distributed with an increasing resistance, presenting a major challenge for the prevention and treatment of GBS [25].

The susceptibility of GBS isolates to the penicillin family supports the first-line antimicrobials in intrapartum chemoprophylaxis against neonatal GBS infection. Vancomycin and linezolid are currently recommended in a second-line role in intrapartum prophylactic treatment regimens against neonatal GBS infection for patients who are allergic to penicillin or suffer from severe infection. In recent years, the increase in natural childbirth has raised the possibility of severe infections in new-born infants with GBS [26, 27]. Notably, the highest rate for tetracycline resistance (90%) in GBS isolates was first found in this region whereas the antimicrobial agents including chloromycetin and levofloxacin were not recommended as antibiotic alternatives for pregnant women or new-borns. The reasons for such resistance to tetracycline, levofloxacin and chloromycetin are unclear and warrant further analysis. It presumes that the pregnant women are liable to drink meat broth and honey to benefit fetal development: tetracyclines and fluoroquinolones are permitted as agents in antimicrobial therapy or use in feed for domestic animals including pig, cattle, goats, and chickens [27]. The chloromycetin, when used for animal growth-promotion, was used to feed bees, resulting in the residue of the drug in Chinese honey. These agents can be transferred from enteric fecal flora in food-producing animals to the pregnant women via the food chain, resulting in higher resistance rates. Additionally, we simultaneously isolated 11 strains from rectal and vaginal swabs, implying the same susceptibility patterns to support our hypothesis. A limitation of the present study was that genotypic data of the GBS isolates were not validated.

In conclusion, the higher prevalence of GBS colonization among pregnant women demonstrated the critical need for screening GBS colonization in late-pregnant women in the region. Also, the study revealed the patterns of resistance of GBS colonization and inferred a possible mechanism of the GBS resistance to antimicrobial agents when used in animal-growth promotion. The appropriate antibiotic prophylaxis can be administrated to prevent GBS transmission from mothers to their new-borns during delivery.

Author contributions

HZ designed the present study. ML performed the assay, and analyzed and interpreted the data. WL wrote the draft manuscript. JL collected samples.

Ethics approval and consent to participate

Human experimental protocols to collect clinical samples were approved by the ethical committees of Beihua University (formal ethical approval number: Protocol Number 2017-10-12). Written informed consent was obtained from all participants in the study.

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Conflict of interest

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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