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Molecular characteristics of *Streptococcus agalactiae* in a mother-baby prospective cohort study: Implication for vaccine development and insights into vertical transmission

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ABSTRACT

Background: *Streptococcus agalactiae* (GBS) is a leading cause of neonatal sepsis and meningitis in many countries. This study aimed to determine the molecular characteristics of GBS colonized in mothers and their infants so as to provide implication for vaccine strategies and confirm vertical transmission.
Methods: A prospective cohort study was conducted to recruit 1815 mother-neonate pairs. All GBS isolates from pregnant women and her infants were tested for serotypes, multilocus sequence types and virulence genes. The relationship between multiple molecular characteristics of GBS isolates was tested by the correspondence analysis, and the agreement between mother-neonate paired data in molecular characteristics was analyzed using Kappa tests.
Results: The predominant serotypes were III, Ia and V, and the most prevalent sequence types (STs) were ST19, ST17, ST10, and ST12. All isolates carried at least one pilus island (PI). The most common combination of PIs was PI-2b alone, followed by PI-1+PI-2a and PI-2a alone, and the most prevalent alpha-like protein (*alp*) genes were *rib*, *epsilon* and *alphaC*. Moreover, a strong relationship was noted between STs, serotypes, *alp* genes and PIs, including ST17 associated with serotype-III/*rib*/PI-2b, ST19 with serotype-III/*rib*/PI-1+PI-2a, and ST485 with serotype-Ia/*epsilon*/PI-2b. The rate of GBS vertical transmission was 14.1%, and the kappa test revealed good agreement in multiple molecular characteristics among GBS-positive mother-neonate pairs. Notably, the switching of molecular characteristics was found during vertical transmission.
Conclusions: Our findings underscore the value of monitoring multiple molecular characteristics so as to provide implication for multivalent strategies and gain insights into GBS vertical transmission and vertical characteristic switching.

1. Introduction

Streptococcus agalactiae, also known as Group B *streptococcus* (GBS), is currently regarded as one of the leading causes of neonatal sepsis, pneumonia, and meningitis worldwide [1]. Many women are asymptomatically colonized with GBS in the digestive and genitourinary tracts [2], but colonized pregnant women are at increased risk of premature delivery and perinatal transmission to their babies [2–5]. It is generally accepted that a neonate acquires GBS isolates by aspiration of the GBS-contaminated amniotic fluid before labor, by vertical transmission during labor, and from the hospital environment after labor [6]. Increasing epidemiological data have suggested maternal GBS colonization as the most predictive factor of GBS infection or colonization in the neonate [7–11]. Notably, although previous studies have defined GBS vertical (mother-to-baby) transmission based on maternal colonization and/or specific molecular characteristics [7–11], few have incorporated multiple molecular characteristics of maternal and neonatal isolates to make transmission evidence more robust. Additionally, few studies have attempted to reveal the vertical characteristic switching based on prospective cohort studies of mother-neonate paired data.

Development of a vaccine is the most promising approach for the prevention of GBS infection given the potential adverse effects of intrapartum antimicrobial prophylaxis as well as the need for

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effective prevention of both neonatal and maternal diseases. The protective role in humans of capsular polysaccharide (CPS)-specific antibodies of GBS has been confirmed for decades [12]. However, the presence of new serotypes and nontypeable isolates and the possibility of shifts in serotypes support the need for ongoing surveillance of serotypes to contribute to the design of a universal vaccine [13,14]. To date, three pilus island (PI) alleles (PI-1, PI-2a, and PI-2b) have been identified on the surface of GBS. Notably, all GBS isolates have either PI-2a or PI-2b present, and many isolates carry the additional PI-1 [15–17]. Identification of these surface exposed antigens that are conserved in the majority of GBS isolates has raised optimism that a pilus-based vaccine can be developed as an alternative [15]. Furthermore, a series of alpha-like proteins (*alp*), encoded by *alphaC*, *rib*, *alp2*, *alp3*, *alp4* and *epsilon* genes, were demonstrated to play an important role in GBS pathogenesis and also regarded as vaccine candidates [7,13,18]. Additionally, the current leading vaccine candidates are protein-CPS conjugate vaccines, and conjugate vaccines composed of the α-C protein and serotype III may be protective against most GBS infections [18,19]. Therefore, accurate population data of surface protein genes and serotype distribution are helpful to provide implication for decision making in GBS vaccine developments.

However, many challenges exist to determine the prevalence and molecular characteristics of GBS isolates, including poor laboratory capacity for diagnosis and genotyping of GBS isolates, especially for low- and middle-income countries. Scarce data are available from China. In order to bridge the knowledge gap, we undertook a mother-baby paired prospective cohort study of GBS colonization. The objective of this study is to determine the molecular characteristics of GBS isolates colonized in mothers and their infants so as to provide implication for vaccine strategies. In addition, this study aimed to confirm GBS vertical transmission and explore the existence of vertical switching of molecular characteristics.

2. Materials and methods

2.1. Study design and population

A prospective cohort study was conducted from July to November 2015 in Shenzhen, China. The study population was pregnant women (≥28 weeks of gestation) who were in labor in the selected hospitals and their newborns. A multistage stratified sampling process was employed to obtain a representative sample. Firstly, all the districts were divided into urban (three districts) and rural (three districts) areas according to geographical representations and levels of economic development. One district was randomly sampled from each of the two categories. Secondly, in each of the two districts, three largest public hospitals having conditions for delivering a baby were included in the sampling frame, and one hospital was randomly drawn from these hospitals. Thirdly, within each selected hospital, all pregnant women and their newborns were sampled to participate in this study, except women with multiple pregnancies.

2.2. Isolation and identification of GBS

Specimens were collected using cotton-tipped swabs. Maternal specimens were obtained from the low vaginal site before labor, and neonatal specimens were taken from the newborn infants (shortly after born in the delivery room) from three sites (including ear, oral cavity and umbilicus). The swabs were enriched in enrichment broth (Trans Vag broth supplemented with 8 μg/ml gentamicin and 15 μg/ml nalidixic acid) at 37 °C in 5% CO_2 for 24 h. To isolate GBS isolates, a loopful of the broth was then plated onto 5% sheep blood agar plates and incubated at 37 °C in 5% CO_2 for 24 h. Strains were confirmed as GBS by β-hemolysis on 5% sheep blood agar plates, gram staining showing gram-positive cocci in pairs or short chains, negative reaction with catalase reagent and positive reaction for the CAMP test.

2.3. Molecular methods

For DNA extraction, the Biospin Bacteria Genomic DNA Extraction Kit (Bioer, Hangzhou, China) was used according to the manufacturer's protocol. All DNA preparations were stored at −20 °C until they were used. The serotypes (Ia, Ib, II-IX) of all GBS isolates were identified by a multiplex polymerase chain reaction (PCR) method as previously described [20]. PCR assays were also used to test the alpha-like protein genes (*alphaC*, *rib*, *epsilon*, *alp2/3*, and *alp4*), pilus-associated genes (*gsb80* for PI-1, *gsb67* for PI-2a, and *san_1519* for PI-2b), adhesion-related gene (*scpB*), and invasion-related gene (*hylB*) [21–23]. Multilocus sequence typing (MLST) of the seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkt*) was conducted using primers and protocols as previously described [24]. The unique sequence of each gene was then uploaded to the GBS database (http://pubmlst.org/sagalactiae/) to provide a unique allele number, and the combination of the allele numbers of the seven loci was given a sequence type (ST). The program eBURST (http://eburst.mlst.net) was used to group isolates into lineages or clonal complexes (CCs), which share six or seven identical alleles. The term "singleton" refers to a ST that did not cluster into a CC. The UPGMA method (MEGA version 6.06) was used to construct the dendrogram to illustrate the pairwise allelic differences and the similarities in molecular characteristics among GBS isolates.

2.4. Statistical analysis

Categorical variables were compared by Pearson's chi-squared test or Fisher exact test when appropriate. The relationships between two or multiple molecular characteristics (such as *alp* gene, PI, serotype and ST) of GBS isolates were tested by the correspondence analysis (simple or multiple). Correspondence analysis provides a useful graphic and statistical method of exploring the internal relationship between categorical variables [25]. In a similar manner to principal component analysis, it provides a means of displaying or summarizing a set of data in two-dimensional graphical form. It is conceptually similar to principal component analysis, but applies to categorical rather than continuous data. The agreement between GBS-positive mother-neonate paired data in molecular characteristics was analyzed using Kappa test. A kappa of 0.4 to 0.75 denotes good agreement, and >0.75 denotes excellent agreement [26]. These analyses were performed using STATA version 14.0 (StataCorp LP, College Station, Texas, USA). A two-sided p value of <0.05 was considered as being of statistical significance.

2.5. Ethics statement

The Ethics Committee of Guangdong Pharmaceutical University approved this study, and the study was performed in accordance with the approved guidelines. Before participating, all pregnant women signed an informed consent form regarding the goals of the study and the willingness to participate.

3. Results

3.1. Participant characteristics

A total of 2192 pregnant women and 1867 infants were willing to participate (Fig. 1). Of these, there were 1815 (82.8%) mother-

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