

Serotyping group B streptococci in a small community hospital: an analysis of distribution and site of isolation

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Objective: To determine the prevalence and site of isolation of different serotypes of group B streptococcus (GBS) colonization or infection at a small community hospital.

Methods: GBS isolates were obtained from a small community hospital and were then serotyped as Ia, Ib, II, III, IV, V or nontypeable. Hospital records were reviewed for patient sex, age and pregnancy status as well as the site of GBS isolation.

Results: GBS serotypes Ia, III and V were most common and accounted for over 60% of the total number of isolates. Serotype Ia was most prevalent in reproductive-age females, while serotypes V and III were most prevalent in non-reproductive-age females and males, respectively. Serotype Ia was most frequent in both pregnant and nonpregnant females. Serotype IV was more common in this study population than in those from other locations.

Conclusions: The GBS serotype distribution in this small community did not differ significantly from distributions described in larger North American centers. A GBS vaccine designed against multiple serotypes would be protective for most of this population.

Key words: AGGLUTINATION; DISTRIBUTION; VACCINE; GROUP B STREPTOCOCCUS

INTRODUCTION

Group B streptococcus (GBS) is the primary bacterial agent causing neonatal infections, including pneumonia, meningitis and sepsis. This organism has also been implicated in adult disease including postpartum infections, urinary tract infections, soft tissue infections and pneumonia¹. While prophylactic antibiotic treatment implemented by many obstetricians has resulted in a significant reduction in infant morbidity and mortality, it does not

address protection of the infant during the first critical months after birth or prevention of adult GBS disease. Vaccine development resulting in the generation of GBS-specific antibody is the strategy of choice. For effective vaccine design, identification of the most prevalent serotypes isolated from the general population is required. In the present study we determined the prevalence of GBS serotypes in a community hospital and compared these data to studies from larger centers in North America.

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SUBJECTS AND METHODS

Study population

Medical records were reviewed from 107 patients who were identified as GBS-culture-positive by the Cabell Huntington Hospital microbiology laboratory (Huntington, WV) from August to November 1999. Patients were classified by retrospective chart review according to sex, age, pregnancy status, previous positive GBS culture and the site of GBS isolation. One hundred and two of the isolates were from females while only five isolates were from males. Patients ranged in age from 18 months to 92 years old. Nonreproductive age females were defined as 18 months to 13 years of age and from 40–92 years of age. Information on race for this population was not available; however, the majority of the communities that are served by this hospital are Caucasian. The most common site of isolation was from genital or urine cultures of reproductive-age females. GBS was also isolated from nonurogenital surfaces including a foot wound, endotracheal tube secretions, blood, a peritonsillar abscess and throat cultures.

GBS isolation

Bacterial cultures obtained from the hospital were streaked on 5% sheep blood tryptic soy agar plates and incubated overnight at 37°C in 5% CO₂ to establish a pure GBS culture. A single colony from each plate was inoculated into Todd Hewitt broth (THB) and incubated overnight at 37°C in 5% CO₂. The overnight cultures were mixed 1:1 (volume:volume) with 24% sterile glycerol and frozen at –20°C until use.

GBS serotype determination

GBS were serotyped using a hemolytic streptococcus group B typing sera kit (Accurate Chemical & Scientific Corporation, Westbury, NY) according to manufacturer's instructions. Briefly, 10 µl of GBS culture from frozen stocks were inoculated into 5 ml THB and incubated overnight at 37°C in 5% CO₂. The overnight cultures were centrifuged at 1400 × *g* for 20 minutes. After discarding the supernatant, the GBS pellet was suspended in 0.5 ml THB. Digestive enzymes from swine

pancreas extracts were added to the GBS suspension prior to incubation at 37°C for 1 hour. The GBS suspension was centrifuged at 1400 × *g* for 20 minutes. The bacterial pellet was suspended in 0.5 ml phosphate-buffered saline (PBS) and incubated at 120°C for 30 minutes. Serotyping of the GBS extract was accomplished by agglutination with serotype-specific antisera (Ia, Ib, II, III, IV or V). PBS was used in place of the antisera as an agglutination control. Agglutination as noted visually or by dark field microscopy was considered a positive serotype result for that isolate. Any extract that failed to agglutinate or demonstrated agglutination in the PBS control was repeated. GBS isolates were labeled as nontypeable (NT) if they failed to agglutinate with any of the antisera or repeatedly agglutinated in all antisera and the PBS control.

Statistical analysis

Statistical analysis was performed using SigmaStat (Jandel Scientific, San Rafael, CA). Chi-square was used for comparisons of serotype frequency between reproductive and non-reproductive-age females, pregnant and nonpregnant females, isolation from the vagina versus the urine and females with vaginitis versus females with no symptoms.

RESULTS

One hundred and seven GBS isolates were obtained from patients at a community hospital in Huntington, WV. The GBS serotype frequencies categorized by patient sex and age, as well as isolation from the vagina or urine, are shown in Table 1. Serotypes Ia, V and III were the most common in this population with frequencies of 23, 21 and 20% respectively. Serotype IV was the least frequent, accounting for only 5% of the total number of GBS isolates. Nine percent of the isolates were nontypeable by agglutination. Restriction fragment length polymorphism analysis on the capsule locus, as described previously by Sellin and colleagues², was attempted to assign serotypes to the NT isolates. However, this method failed to provide any additional information (data not shown).

Table 1 GBS serotypes by patients' sex, age and site of isolation

| Serotype | Males | Females | | | | | Percent of total |
|----------|-------|-------------|-------|------|----------------|---------|------------------|
| | | Age (years) | | | Isolation site | | |
| | | < 13 | 13–39 | > 39 | Vagina* | Urine** | |
| Ia | 1 | 0 | 21 | 3 | 19 | 5 | 23 |
| Ib | 1 | 2 | 11 | 1 | 13 | 1 | 14 |
| II | 0 | 0 | 8 | 1 | 9 | 0 | 8 |
| III | 2 | 2 | 15 | 2 | 15 | 3 | 20 |
| IV | 0 | 0 | 4 | 1 | 4 | 1 | 5 |
| V | 1 | 1 | 14 | 6 | 15 | 4 | 21 |
| NT | 0 | 0 | 5 | 5 | 9 | 1 | 9 |

*GBS were isolates from swabs taken from the lower third of the vagina and the rectum for pregnant females, while swabs were taken from the vagina only for symptomatic, nonpregnant women; **urine specimens were considered positive for GBS when > 10 000 colony-forming units were present; NT, nontypeable

The majority of isolates were obtained from reproductive-age women (73%) while males accounted for only 5% of the total. In the male population, serotype III was most commonly isolated. No statistical comparisons between the female and male serotype distributions were made due to the small number of GBS isolates from males ($n = 5$). In reproductive-age females, serotype Ia was the most frequent, while serotype V was most frequent in non-reproductive-age females. However, the individual serotype frequencies between these two groups were not significantly different ($p = 0.273$) (Table 1). Additional comparisons made with respect to pregnancy status ($p = 0.239$), site of isolation (vaginal versus urine; $p = 0.71$), and symptomatic vaginitis (versus asymptomatic females; $p = 0.511$) revealed no significant differences.

Eleven percent of the women in this study had infants with neonatal intensive care unit visits. None of these infants was under observation for GBS infection. All six GBS serotypes were isolated from these women; however, serotypes Ib, II and V were most frequent with three isolates each. In addition, the five females with previous positive GBS cultures were also carrying isolates of the most common serotypes: Ia ($n = 2$); III ($n = 1$); and V ($n = 2$).

DISCUSSION

It has been well documented that antibodies to GBS-serotype-specific capsule polysaccharide are protective^{3,4}. Unfortunately, few individuals generate sufficient quantities of these antibodies. Recent attempts at designing a vaccine against serotype III GBS yielded less than ideal results with only 40% of women vaccinated generating antitype III antibodies⁵. Protein carriers, including the tetanus toxoid⁶, are being evaluated for their ability to enhance the immunogenicity of the GBS capsule polysaccharide. Although such a protein-polysaccharide conjugate vaccine shows promise, a vaccine against multiple GBS serotypes will be most efficacious. Harrison and colleagues² report that a pentavalent vaccine against serotypes Ia, Ib, II, III and V would protect approximately 95% of their study population.

The optimal design for a multivalent GBS vaccine will include the most common capsule polysaccharide serotypes. To date, much of the data regarding GBS serotype distribution has been obtained from large centers. The purpose of our study was to determine the serotype frequencies in a small community hospital in West Virginia and to compare our data to those from larger North American centers.

Table 2 Comparison of GBS serotype frequencies between Huntington, WV and other North American populations

| Serotype | Positive GBS frequency (percent) | | | | | |
|----------|----------------------------------|-------------------|-----------------|--------------------|-----------------------|-----------------------|
| | WV | MD ^{7,*} | IA ⁸ | TX ^{9,**} | Alberta ¹⁰ | Calgary ¹¹ |
| Ia | 23 | 28 | 25 | 24 | 17 | 22.7 |
| Ib | 14 | 12 | 6 | 9 | 10 | 10.7 |
| II | 9 | 12 | 5 | 26 | 9 | 12.9 |
| III | 20 | 16 | 52 | 24 | 19 | 20.6 |
| IV | 5 | < 1 | 0 | 0 | 0 | 2.1 |
| V | 21 | 26 | 9 | 12 | 31 | 19.3 |
| NT | 9 | 4 | 3 | 4 | 13 | 11.2 |

*Values represent data on adults compiled from Table 1 in Harrison et al⁷; **Values represent data compiled from Table 2 in Hickman et al⁹; WV, West Virginia; MD, Maryland; IA, Iowa; TX, Texas; NT, nontypeable

Comparison of the West Virginia serotype frequencies with those observed primarily in adults or pregnant women from Maryland⁷; Iowa⁸; Texas⁹; Alberta, Canada¹⁰; and Calgary, Canada¹¹ is shown in Table 2. The three most common serotypes in our study and the others are Ia, III and V with the exception of Texas where Ia, II and III were the most prevalent. While serotype IV was least common in all populations, in our study it accounted for 5% of the total number of isolates, which differs from the other study populations that report frequencies of 0–2%. Although this difference is slight, it might indicate that serotype IV GBS is an emerging serotype in our community. This trend should be monitored in the future as a shift in the serotype distributions may alter efficacy of a potential vaccine. Nontypeable isolate frequencies ranged from 3 to 13% in the large North American centers. Our West Virginia population was 9% nontypeable.

Of the centers with which we compared our data, our population most closely resembles those from Canada. Yet overall the serotype distribution in our West Virginia community does not differ greatly from any of the larger centers. As such, the pentavalent vaccine proposed by Harrison² would be effective in protecting at least 86% of our population. Based on this study, we are confident that our community is similar enough to large metropolitan areas that a multivalent vaccine designed from serotype frequencies in these communities would also be effective for our area.

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