Invasive Group B Streptococcal Disease: The Emergence of Serotype V

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Group B streptococci (GBS) cause invasive disease in neonates, pregnant adults, and nonpregnant adults with underlying or chronic disease. Previous studies found capsular serotypes Ia, Ib, II, and III cause invasive disease. Prospective population-based surveillance of invasive GBS disease was done from June 1992 to June 1993 in metropolitan Atlanta: 279 patients had invasive disease. Of these, 43% were <6 months old, and 57% were adults. The incidence among all adults was 7.7/100,000/year, 33% higher than in 1989–1990 (P < .01); the incidence in nonpregnant adults was 5.9/100,000/year, 37% higher than in 1989–1990 (P < .02). Serotyping of 178 patient isolates revealed that 34% had GBS serotype Ia or Ia/c, 8% had Ib/c, 6% had II or II/c, 29% had III, 0% had IV, 21% had V, and 2% were nontypeable. Serotype V was recovered from all groups and was the most common serotype from nonpregnant adults. Serotype V isolates appeared to be highly related genetically. The increasing incidence of GBS disease in adults, the changing distribution of serotypes, and the emergence of serotype V will impact vaccine strategies.

Resistance to infection with GBS has been correlated with the presence of serum antibodies to type-specific determinants of capsular polysaccharides in both experimental animals and human neonates [10,11]. Ongoing efforts to develop efficacious vaccines for the prevention of GBS disease in humans will require precise knowledge about serotype distribution and groups at risk for infection.

Previous studies have reported a distribution of capsular serotypes Ia, Ib, II, and III, with few nontypeable isolates in neonates with early-onset disease (i.e., GBS disease occurring in the first week of life) and among pregnant women with vaginal GBS colonization [2,12–14]. Late-onset neonatal disease (i.e., disease occurring in newborns >7 days of age) and meningitis among neonates is due primarily to serotype III, which accounts for 70% of such cases [15]. Little is known about serotype distribution or genetic relatedness of GBS isolates recovered from nonpregnant adults with invasive disease.

Using prospective population-based surveillance, we explored the incidence of GBS disease among all age groups, the distribution of serotypes among GBS isolates recovered from those with invasive disease, including nonpregnant adults, and the molecular epidemiology of GBS disease in metropolitan Atlanta.

Materials and Methods

Population-based surveillance. We did a prospective population-based surveillance of invasive GBS disease in the eight-county metropolitan Atlanta area from 1 June 1992 through 30 June 1993. The population of the surveillance area was 2,460,233 during the study period (Georgia Department of Human Resources, Atlanta). About 68% of the residents were white, 29% were black, and 3% were of other races or ethnic groups (1990 US census data). During the study period, there were 44,473 live births in the surveillance area (Georgia Department of Human Resources).
Infections were diagnosed on the basis of isolation of GBS from normally sterile sites (e.g., blood, peritoneal fluid, or cerebrospinal fluid). All patients with invasive GBS disease were living in the surveillance area during the study period. Patients with urinary tract infections and those with placental isolation were not included unless they had bacteremia. GBS isolates were collected from 32 hospitals and 1 referral laboratory. Strains were identified as GBS at local hospital laboratories with standard, commercially available diagnostic kits. All kits were based on the extraction of streptococcal group antigens in soluble form and their identification with use of latex particles coated with group-specific antibody. Isolates collected from hospital clinical microbiology laboratories were subcultured and incubated overnight. GBS isolates were then stored at −70°C in sheep’s blood until serotyping and molecular typing studies were done.

Initial case reports of invasive GBS disease were obtained from two independent sources: hospital microbiology laboratories and hospital infection control practitioners. Every 6 months during the study period, laboratory audits were done at all hospitals to evaluate reporting accuracy and to identify any cases that had not been reported. Demographic information (including age, sex, race, and pregnancy status) and clinical findings were obtained for all patients.

Serotyping. Serotyping was done by the Lancefield capillary precipitin method [16]. Antisera to polysaccharide antigens Ia, Ib, II, III, IV, and V and protein antigen c were used. Antisera were prepared at the Centers for Disease Control and Prevention (CDC; Atlanta).

Molecular typing studies. DNA restriction fragment length polymorphism (RFLP) of rRNA genes (ribotyping) and restriction endonuclease analysis of chromosomal DNA (REAC) were done as previously described [17]. Chromosomal DNA was extracted as described by Pitcher et al. [18] after organisms were grown overnight in Todd-Hewitt broth at 37°C with aeration. DNA (3 μg) was digested by HhaI (1 μL or 10 U) for 2 h at 37°C in a 20-μL reaction mixture according to manufacturer’s recommendations (New England Biolabs, Beverly, MA). An additional 1 μL of restriction enzyme was added, and the reaction mixture was reincubated for 2 h. HhaI was previously shown to provide the best discrimination of hybridization banding patterns among 24 restriction enzymes studied [17]. The digested DNA was electrophoresed in a 1% agarose horizontal gel at 60 V for 18 h in TRIS-acetate buffer. After electrophoresis, gels were stained in ethidium bromide and photographed under UV light. A 1-kb ladder and λ phage (GIBCO BRL, Gaithersburg, MD) were used as molecular size standards. Digested and electrophoresed DNA restriction fragments were denatured and transferred to a nylon membrane (magna-graph, MSI, Westboro, MA) by the method of Southern [19]. Escherichia coli 16S and 23S rRNA (Boehringer Mannheim Biochemicals, Indianapolis) were used for the probe. Hybridization experiments were done with a nonradioactive labeling system (Genius system; Boehringer Mannheim) as previously described [20, 21].

Pulsed-field gel electrophoresis (PFGE) was done on 47 GBS isolates; whole cell DNA was prepared as described by Murray et al. [22] with the following modifications: Mutanolysin (1 μg/mL; Sigma, St. Louis) was added to the bacterial lysis solution, and whole cell DNA was digested with Smal (Boehringer Mannheim). PFGE was done using the contour-clamped homogeneous electric field method; pulse time was increased from 1 to 30 s over 23 h at 200 V. A λ ladder PFGE marker (Boehringer Mannheim) was used as a molecular size standard. PFGE banding patterns were compared visually; strains were considered distinguishable if PFGE patterns differed by ≥3 bands [23]. Banding patterns were also compared by use of a computer-assisted system in which DNA typing gels were analyzed using Bio Image/Millipore whole band analyzer software (Bio Image, Ann Arbor, MI). Cluster analysis using the method of unweighted pair–group average was done to calculate similarity or dissimilarity among GBS isolates. A significant difference was defined as a coefficient of similarity of <80%.

Statistical analysis. Incidence rates of invasive GBS disease in the eight-county area during the study period were compared to those previously reported [5] for the same area in 1989–1990 using Fisher’s exact test. The surveillance area and methodology used during both time periods were identical. Incidence rates of GBS disease in black and in white patients during the study period were compared using the χ² test. P < .05 was considered significant.

Results

Over 13 months (1 June 1992 to 30 June 1993), there were 279 cases of invasive GBS disease in metropolitan Atlanta. Overall, 112 (40%) of the cases occurred among nonpregnant adults, 48 (17%) among pregnant adults, 90 (32%) among neonates <7 days of age (early-onset neonatal disease), and 31 (11%) among infants ≥7 days to <6 months old (late-onset neonatal disease). There were no cases in persons 6 months to 15 years old. Of the 119 neonatal cases, 76% were due to early-onset and 24% to late-onset neonatal disease. Of the 160 adult cases, 30% were pregnancy related and 70% occurred in nonpregnant adults. The mean age of the adults with nonpregnancy-related GBS was 57.2 ± 18.1 years, and 53% were men. Of the 279 people with GBS infection, 53% were black, 44% white, and 3% were of other races or ethnic groups. The incidence of invasive GBS disease was significantly higher among blacks than for whites for all age groups (P < .05) except the 5-to-14-year-old group, in which there were no cases of invasive GBS disease (figure 1).

The overall incidence of invasive GBS disease during the study period was 10.5/100,000 residents/year; this was a 14% increase from 1989–1990, but the difference was not statistically significant (P = .1; table 1). The incidence among all adults was 7.7/100,000/year during the study period, which was 33% higher than in 1989–1990 (P < .01). This increase among all adults (≥15 years old) was due to the increase in GBS cases among nonpregnant adults. There were 5.9 cases/100,000/year among nonpregnant adults, during the study period, 37% more than in 1989–1990 (P < .02). There were 2.7 cases/1000 live births, a number similar to that seen in 1989–1990 (table 1).

Serotyping. GBS isolates from 178 (64%) of the 278 patients were available for further study. Serotyping and molecular typing studies were done on these isolates (81 from infants, 65 from nonpregnant adults, and 32 from pregnant adults). The
distribution of GBS serotypes is shown in table 2. Overall, 34% of the isolates were serotype Ia or Ia/c, 8% were Ib/c, 6% were II or II/c, 29% were III, 0% were IV, 21% were serotype V, and 2% were not typeable. Serotype V isolates were recovered from all age groups (neonates, pregnant adults, and nonpregnant adults). The proportion of GBS cases due to serotype V ranged from 11% in the neonates to 31% among the nonpregnant adults. Serotype V and Ia or Ia/c were the most common serotypes associated with invasive GBS disease among nonpregnant adults (table 2).

Molecular epidemiologic typing. One hundred seventy of the GBS isolates were evaluated by restriction endonuclease analysis of chromosomal DNA (REAC) and restriction fragment length polymorphism of rRNA genes (ribotyping) (figures 2, 3). Overall, 13 different hybridization banding patterns (ribotypes) were noted among the 170 GBS isolates studied; 10 of the ribotypes are shown in figure 2B. Three different ribotype patterns were noted among the 58 Ia or Ia/c isolates, 1 ribotype among 13 serotype Ib/c isolates, 6 ribotypes among 11 serotype II or II/c isolates, 5 ribotypes among 47 serotype III isolates, and a single ribotype (pattern 3) among all 37 serotype V isolates studied. The distribution of ribotypes is shown in table 3. Patient age or race did not affect the distribution of ribotypes (data not shown). All serotype Ib/c and V isolates and 3 serotype II/c isolates had ribotype pattern 3 (table 3). Serotype V isolates had identical or very similar REAC patterns; the REAC pattern observed with serotype V isolates clearly differed from that seen with serotype Ib/c isolates or ribotype 3, serotype II/c isolates (figure 3).

Forty-seven GBS isolates (33 serotype V and 14 of other serotypes) were also studied by PFGE (figure 4). Twenty-five of the 33 serotype V GBS isolates had the same PFGE banding pattern, and all 33 serotype V GBS isolates were highly related, having a coefficient of similarity of >80% (figure 4A). The serotype V isolates were clearly different than other GBS serotypes studied by PFGE; the similarity coefficient between the serotype V isolates and those of other serotypes studied was 50% (figure 4B). PFGE clearly differentiated between isolates of different serotypes (V, Ib/c, and II/c) that had ribotype pattern 3.

Discussion

GBS infections have emerged as an important cause of morbidity and mortality among nonpregnant adults as well as neonates and pregnant or postpartum women [5, 7]. Our study indicates that the incidence of invasive GBS disease among

![Figure 1. Incidence of invasive GBS disease in metropolitan Atlanta by age and race.](https://academic.oup.com/jid/article-abstract/173/2/365/896048)
Table 2. Distribution of GBS serotypes.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Overall</th>
<th>Neonates</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Ia, la/c</td>
<td>60 (34)</td>
<td>23 (36)</td>
<td>4 (23)</td>
</tr>
<tr>
<td>Ib/c</td>
<td>15 (8)</td>
<td>6 (9)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>II, II/c</td>
<td>11 (6)</td>
<td>5 (8)</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>51 (29)</td>
<td>19 (30)</td>
<td>12 (71)</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>37 (21)</td>
<td>9 (14)</td>
<td>0</td>
</tr>
<tr>
<td>NT</td>
<td>4 (2)</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Total no.</td>
<td>178</td>
<td>64</td>
<td>17</td>
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</table>

NOTE. Data are no. (%) of patients. NT = nontypeable; early = early-onset neonatal disease (among infants <7 days old); late = late-onset neonatal disease (among infants ≥7 days of age).

Figure 2. A. Restriction endonuclease analysis of chromosomal DNA patterns of selected GBS isolates. Lane nos. correspond to those in B. B. Corresponding hybridization banding patterns (ribotypes). Serotypes and ribotype pattern numbers are at bottom. By lane, serotype, ribotype, and isolate designations are I, Ia/c, 1, and 400; 2, Ia/c, 2, and 404; 3, Ia/c, II, and 332; 4, Ia/c, 3, and 321; 5, V, 3, and 380; 6, II/c, 5, and 572; 7, II/c, 12, and 406; 8, III, 6, and 441; 9, III, 7, and 368; 10, III, 8, and 324; and 11, III, 10, and 390. M, 1-kb ladder (molecular size marker).
adults continues to increase in metropolitan Atlanta, primarily due to an increase of disease among nonpregnant adults. In our prospective population-based study, there was a 37% increase in the incidence of invasive disease among nonpregnant adults in 1992–1993 (to 5.9 cases/100,000/year) compared with a similar study in 1989–1990 [5]. In fact, the incidence of invasive GBS disease appears to have more than doubled over the past decade in metropolitan Atlanta. A population-based retrospective survey by Schwartz et al. [6] done in metropolitan Atlanta in 1982–1983 estimated the annual incidence of invasive GBS disease among nonpregnant adults to be 2.4/100,000.

The reasons for the continuing increase in disease are unclear, although one factor may be longer survival of adults with underlying chronic diseases who are at greatest risk for GBS infection [7]. A 34% increase in the incidence of pregnancy-related GBS disease among women during 1992–1993 compared with 1989–1990 was also seen, but the difference did not achieve statistical significance (P = .14). There appears to have been a marked increase in the incidence of pregnancy-related disease in metropolitan Atlanta over the past decade, as we noted a 3-fold increase in the incidence of pregnancy-related adult disease compared with that reported by Schwartz et al. [6] (66.4 vs. 22/100,000 pregnancies). The incidence of neonatal or infant cases (2.7/1000 live births) during our study period was unchanged from that in 1989–1990. It remains to be determined whether recently published strategies (i.e., intrapartum chemoprophylaxis of high-risk maternal carriers) to prevent infection of the neonate [1, 24, 25] will influence rates of neonatal infection in metropolitan Atlanta. A survey done in August 1993 of 192 physicians suggested that many Georgia physicians who provide obstetric care were not using recommended practices to prevent perinatal GBS disease [26].

In our surveillance area, blacks account for 29% of the population, but over half of all patients with invasive GBS disease were black. The incidence of GBS infection was higher among blacks than whites for all age groups (i.e., infants and pregnant and nonpregnant adults). Our findings are consistent with those
of previous studies, which also noted higher rates of GBS infection among blacks [5, 6, 27, 28]. The reasons for higher rates of GBS disease among blacks than whites are incompletely understood. Differences in the incidence of disease may reflect socioeconomic conditions or potentially higher rates of chronic disease among nonpregnant black adults. Higher rates of pregnancy-related and neonatal disease among blacks may also be related to higher rates of vaginal colonization. Regan et al. [29] found a higher prevalence of GBS vaginal colonization in black (~21%) than in white (~14%) pregnant women. Other smaller studies, however, have reported no differences in GBS prevalence by ethnic group [30, 31].

Despite structural similarities, the GBS capsular type polysaccharides (Ia, Ib, II, III, IV, V, and VI) are immunologically distinct, and mechanisms of host protection are believed to be based in large part on recognition by antibodies of capsular antigen specificity. Antisera raised to GBS type-specific capsular antigens are protective against experimental GBS infection, and in human disease, type-specific antibodies correlate with protection in neonates [10]. In contrast, antisera to the common group B antigen fail to protect in animal models of lethal GBS infection, and the level of naturally occurring common group B antibody correlates poorly with resistance to human infection [10, 32, 33]. Current research is focused on the

Figure 4. A, Dendrogram of 47 GBS isolates studied by pulsed-field gel electrophoresis (PFGE) showing degree of relatedness (coefficient of similarity). Serotype, lane no. corresponding to lanes in B, and no. of isolates with particular PFGE banding pattern are at right. B, Representative PFGE patterns of GBS. Lanes: 1–8 are serotype V (isolates 576, 360, 385, 472, 392, 402, 380, and 526, all ribotype 3); 9–11 are Ia/c (isolate 453, not ribotyped; isolates 442 and 440, ribotypes 1 and 2, respectively); 12 and 13 are II/c (isolates 408 and 437, ribotype 3); 14 is II (isolate 345, ribotype 8); 15 is III (isolate 377, ribotype 8); 16 and 17 are III (isolates 396 and 227, ribotype 6); 18 is II (isolate 517, ribotype 5); 19 is II/c (isolate 406, ribotype 12); and 20–22 are Ib/c (isolates 426, 337, and 340, ribotype 3).
development of type-specific polysaccharide-protein conjugate vaccines. Encouraging results in immunogenicity have been obtained for capsular polysaccharide–protein conjugate vaccines of serotypes Ia, II, III, and, recently, serotype V in the prevention of GBS infection in a mouse animal model [11, 34, 35].

Knowledge of the distribution of serotypes is essential for developing and formulating a protective human GBS vaccine. Our data suggest that there has been a significant shift in the distribution of GBS serotypes causing invasive disease and that serotype V has emerged as a major cause of disease. Studies done more than a decade ago indicated that neonatal disease was caused by serotypes Ia, Ib, II, and III, with few nontypeable isolates [2, 12–14]. Little has been known about serotype distribution among adults, especially among nonpregnancy-related cases. In our study, serotype V isolates were seen among all age groups except neonates with late-onset disease. Serotype V isolates accounted for 21% of the 178 GBS isolates examined (14% in neonates with early-onset disease, 25% in pregnant adults, and 31% in nonpregnant adults). Serotype V was the most common serotype recovered from nonpregnant adults with invasive GBS disease and the second most commonly recovered serotype from pregnant adults. Clearly, an effective capsular polysaccharide vaccine to prevent disease among neonates and pregnant and nonpregnant adults should be multivalent and provide protection against serotype V disease.

Serotype V was first reported by Jelinkova and Motlova [36] in 1985; it was identified among a collection of previously nontypeable GBS isolates recovered between 1960–1981, including nontypeable US isolates collected by Wilkinson [37]. Type-specific antibodies to serotype V isolates do not cross-react with other GBS capsular types and appear to recognize an epitope that is not as heavily dependent on the presence of

### Table 3. Distribution of hybridization banding patterns (ribotypes).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Ribotype pattern*</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Ia, la/e</td>
<td>1</td>
<td>36</td>
</tr>
<tr>
<td>Ia, la/e</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Ia/c</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Ia/c</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>II/e</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>II/e</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>II/e</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>II/e</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>II/e</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
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<tr>
<td>Ib/e</td>
<td>ND</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>ND</td>
<td>4</td>
</tr>
</tbody>
</table>

NOTE. NT = nontypeable; ND = not done.

* Ribotype pattern indicates hybridization banding pattern as shown in figure 2B.
the terminal side chain sialic acid residues as that of type Ia, II, and III GBS polysaccharides [38]. Serotype V isolates have been recovered from a number of countries around the world [31, 39–42]; however, these reports suggested that serotype V was a distinctly uncommon serotype. There have been a few recent clinical case reports that have described invasive GBS disease due to serotype V in neonates with early- or late-onset disease in the United States and Europe, and there has been a report of invasive disease in one nonpregnant adult [43–46]. In addition, a recent brief report indicated that serotype V disease had emerged in Maryland and accounted for 9 (12%) of 77 GBS isolates recovered from neonates and 44 (31%) of 141 isolates recovered from adults with invasive GBS disease in 1991–1993 [47]. Our findings on the proportion of serotype V isolates causing invasive GBS disease in Atlanta are similar to those reported from Maryland.

It is uncertain when, how, and why serotype V emerged, but it may have been a common serotype in the United States since at least the mid-1980s. Serotyping studies on isolates recovered from neonates in the 1970s rarely demonstrated isolates that were nontypeable. However, a prospective laboratory-based surveillance project by the CDC in 1986 in California, Missouri, New Jersey, Oklahoma, Tennessee, and Washington recovered a number of GBS isolates that were nontypeable by antisera to serotypes Ia, Ib, II, and III (17 [24%] of 72 isolates recovered from nonpregnant patients >6 months of age, 5 [12%] of 43 from pregnant patients, and 10 [8%] of 110 from patients with early-onset neonatal disease) [15]. Many of the nontypeable isolates from that CDC project have recently been demonstrated to be serotype V isolates (unpublished data). In addition, a number of GBS isolates recovered from nonpregnant adult patients at Grady Memorial Hospital (Atlanta) in 1987–1988 that were nontypeable with antisera to serotypes Ia, Ib, II, and III have recently been confirmed as serotype V (unpublished data).

Molecular analyses of GBS using REAC and ribotyping or PFGE have proven to be useful subtyping methods and have provided the ability to discriminate among isolates of the same serotype [17, 48–50]. In our study, we noted 13 different ribotype patterns among the 170 isolates studied; 159 isolates were of six different ribotype patterns. As noted previously [17], these methods were very reproducible and REAC appeared to be a more sensitive typing method than ribotyping. PFGE also proved to be more sensitive than ribotyping, and because fewer bands are produced, it is much simpler than REAC to analyze. All serotype V isolates studied had the same ribotype (pattern 3) and the same or similar REAC patterns, and the 33 serotype V isolates studied by PFGE had the same or similar PFGE banding patterns (coefficient of similarity of >80%), suggesting that serotype V GBS isolates recovered from patients in our surveillance area are highly related. PFGE and REAC clearly differentiated between serotype V isolates and isolates of other serotypes (Ib/c and II/c) that had ribotype hybridization banding pattern 3. Molecular analysis of serotype V isolates from other geographic areas will be needed to definitively determine if all serotype V isolates are highly related.

In summary, the incidence of invasive GBS disease (particularly among nonpregnant adults) continues to increase in the Atlanta area. Serotype V has emerged as a major cause of disease among all age groups and especially among adults. Serotype V isolates recovered from patients with invasive disease appear to be highly related genetically, suggesting the dissemination of closely related strains. The changing distribution of serotypes and emergence of serotype V will have a major impact upon vaccine development.

Acknowledgments

We thank J. William Eley for assistance with statistical calculations, Susan Hunter for assistance with cluster analysis, and the hospitals of Georgia Health District III and the staff of the Atlanta Active Surveillance Project for help and cooperation.

References


